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Novel secondary preventative strategies in the management of ischaemic stroke and transient ischaemic attack

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Abstract

Stroke represents the second leading cause of global mortality. Despite improvements in acute stroke management in recent years, many patients suffer a poor outcome and stroke represents a leading cause of adult disability. Beyond the human impact of disease, societal costs are huge. Stroke is principally a disease of the elderly population, which is increasing in size. Incidence is increasing in the younger population and the developing world. Accordingly, the global burden of stroke is anticipated to increase substantially in the coming decades.

Fatal and disabling strokes are frequently preceded by either a milder, non-disabling stroke or a transient ischaemic attack (TIA). The optimised implementation of effective preventative strategies and the evaluation of potentially novel therapeutic agents, both represent crucial strategies in reducing the burden of cerebrovascular disease. This thesis addresses two aspects of secondary prevention following ischaemic stroke: Firstly, improved identification and optimised use of anticoagulant based secondary prevention for an established but frequently occult risk factor for recurrent stroke; paroxysmal atrial fibrillation (PAF). Secondly, the evaluation of xanthine oxidase inhibition (XOI), as a potential therapeutic strategy for the prevention of stroke and cardiovascular disease.

Chapter 1 highlights the importance of optimising secondary preventative strategies following ischaemic stroke and TIA. The anticipated increase in the global burden of cerebrovascular disease is described, together with review of stroke outcomes in the context of contemporary acute stroke management. The risk of stroke recurrence following ischaemic stroke and TIA is reviewed, followed by discussion of available secondary preventative strategies.

In Chapter 2, the role of AF in the aetiology of stroke disease is reviewed in greater detail. The early and subsequent risk of stroke recurrence in patients with AF is considered, together with review of the evidence for antithrombotic therapy. The potential for increased detection of paroxysmal AF in the ischaemic stroke and TIA population is discussed, with particular reference to the therapeutic implications and potential for reduced stroke recurrence.

In Chapter 3, details of a randomised, open-label trial with objective outcome assessment, in patients with recent (< 7 days) ischaemic stroke or TIA, are presented. This study evaluated the use of non-invasive cardiac event monitoring to detect AF, in patients with ischaemic stroke or TIA presenting with sinus rhythm, an area of management for which a robust evidence base is lacking. The primary endpoint was the difference in AF detection at 14 days, between patients randomised to receive supplemental cardiac event monitoring of 7 days duration, compared to those receiving standard guideline based investigation alone. Detection of AF was defined as evidence of “sustained” PAF and “any duration” PAF episodes. Secondary endpoints were the difference in AF detection at 90 days and the difference in AF-thromboembolic prophylaxis-related anticoagulation (e.g. warfarin) at 14 & 90 days. 100 patients were enrolled and completed 90 day follow up. “Sustained” and “any duration” PAF episodes were detected, respectively, in 16% (95% CI, 7.2 – 29.1%) and 42% (95% CI, 28.2 – 56.8%) of patients through 7 days non-invasive cardiac event monitoring. Supplementation of standard guideline based investigation with 7 days’ cardiac event monitoring resulted in detection of “sustained” and “any duration” PAF episodes in an additional 16% (4.7 – 27.3%) and 40% (25.2 – 54.8%) of patients, respectively, at 14 days follow up. These differences persisted at 90 days. Anticoagulant therapy was commenced within 14 days in 16% of the group randomised to receive supplemental monitoring, versus none randomised to standard investigation alone ($p < 0.01$). This difference persisted to 90 days (22% versus 6%, $p < 0.05$). This study provided the first randomised controlled evidence for a specific investigation strategy for the detection of occult PAF in unselected patients with ischaemic stroke and TIA. These data should support guidelines regarding the management of this patient group. In addition, the study provided evidence that this strategy is not reliant on specialist electrocardiological service support, with very good agreement [(96%; 95% CI, 86.3 – 99.5%), Fleiss’ Kappa 0.86, $p < 0.00001$] observed between non-cardiology trained stroke clinicians and the independent reporting electrocardiology laboratory for “sustained” PAF detection.

In a related observational study, the predictive value of detection of AF in the period immediately following ischaemic stroke or TIA was examined, relative to interval evaluation for AF by non-invasive cardiac event monitoring, performed 90 days following the index event. This study demonstrated that identification of AF immediately following stroke holds

strong positive predictive value for subsequent AF detection following a 90 day interval, supporting treatment decisions being based on the findings of early monitoring. Negative predictive value of failure to identify AF through early monitoring was only modest, suggesting that repeated investigation may be of value.

In Chapter 4, the hypothetical role of uric acid (UA) in the pathogenesis of cardiovascular disease is reviewed. Conflicting data are discussed, including those which support the hypothesis that serum UA represents a surrogate marker of elevated cardiovascular risk attributable to either its association with established cardiovascular risk factors or, alternatively, as a surrogate marker for a novel mechanism of vascular injury: harmful oxidative stress produced by the xanthine oxidase enzymatic system. XO is discussed as a potential novel therapeutic strategy in the prevention and management of cardiovascular disease.

In chapter 5 a systematic review and meta-analysis of studies evaluating the effect of XO on cardiovascular health in humans are presented. This study identified a body of heterogeneous studies, reporting largely positive results. In meta-analysis, improvement in brachial artery flow mediated dilatation, [2.50% increase (95% CI, 0.15 – 4.84%)] and venous occlusion plethysmography derived forearm blood flow response to acetylcholine, [69% increase relative to control arm (95% CI, 18 – 119%)], indicated that XO improves endothelial function amongst patients with, or at risk of, cardiovascular disease. In addition, XO reduces vascular oxidative stress, Malondialdehyde reducing by 0.56 nmol/ml (95% CI 0.26 – 0.87 nmol/ml). Several studies indicated that the benefits of treatment may be limited to patients with hyperuricaemia. Definitive clinical endpoint studies were lacking. The study confirmed that XO has beneficial effects within the cardiovascular system, providing supportive evidence for further study in the stroke patient population and in relation to more definitive clinical endpoints.

In Chapter 6, three surrogate measures of cardiovascular risk: Carotid intima media thickness (CIMT); blood pressure pulse wave analysis (BP-PWA); and endothelial function are reviewed. Each is considered with respect to their independent predictive value for risk of cardiovascular disease, and utility as surrogate outcome measures in clinical trials. Together

with chapter 4 and the systematic review reported in chapter 5, this chapter provides additional background to the randomised controlled trial reported in chapter 7.

In Chapter 7, the details of a double blind, randomised placebo-controlled trial, evaluating XOI (with Allopurinol 300 mg once daily) in a population with recent (< 12 months) ischaemic stroke or TIA, are presented. Patients were followed up at 1, 3, 6 & 12 months in relation to several prognostically important surrogate measures of cardiovascular function. End points included differences in CIMT progression, change in central blood pressure (BP) and change in arterial stiffness at one year and change in endothelial function at 6 months. Data regarding safety and tolerability were also collected. Eighty participants were recruited, mean age 67.8 (SD 9.4) years. Progression in mean common CIMT at one year was less in allopurinol treated patients compared with placebo [between group difference, -0.097mm (95% CI, -0.175, -0.019), $p=0.015$]. Mean maximum CIMT progression did not differ between treatment groups at one year [between group difference, -0.060mm (95% CI, -0.146, 0.027), $p=0.17$]. Central aortic systolic BP [-6.6 mmHg (95% CI -13.0 to -0.3), $p=0.042$] and augmentation index [-4.4% (95% CI -7.9, -1.0), $p=0.013$], were each reduced following allopurinol treatment compared with placebo at 12 months. No significant difference was observed for measures of endothelial function. This study extends the evidence of sustained beneficial effects of allopurinol on the vasculature to the stroke population and to endpoints including CIMT progression and central BP. The study confirmed that a previously reported benefit of reduced arterial stiffness represents a sustained treatment effect. These data provide evidence in support of the hypothesis that the therapeutic strategy of XOI has the potential to reduce the risk of cardiovascular events.

The studies presented in this thesis provide a combination of clinically relevant and novel scientific findings. The two randomised controlled clinical trials were robustly conducted studies, adhering to vigorous regulatory standards and provide prospective data obtained in clinical patient populations. The evidence from the non-invasive cardiac event monitoring study should immediately support the optimisation of care of patients with acute ischaemic stroke or TIA for the detection of occult paroxysmal AF, thereby optimising use of anticoagulant therapy. The systematic review and clinical trial evaluation of XOI, respectively, provide confirmatory evidence of beneficial effects within the vasculature and

extend this to the stroke patient population, including to the surrogate markers of CIMT progression and central BP. Together they provide supportive evidence for more definitive study to evaluate whether XOI reduces the risk of cardiovascular disease.

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Declaration

The work included in this thesis was performed during and subsequent to my period as a Clinical Research Fellow in the University of Glasgow Institute of Cardiovascular & Medical Sciences at the Western Infirmary, Glasgow.

The non-invasive cardiac event monitoring study was competitively funded by a grant from the Chief Scientist Office, Scotland (CZG/2/745) and supported by the Scottish Stroke Research Network. The funder did not contribute to study design, study conduct, report preparation or submission.

Six “R-test Evolution 3” cardiac event monitors and accompanying software for rhythm analysis, required for conduct of the studies reported in Chapter 3, were donated to the Western Infirmary Acute Stroke Unit by Novacor, who also provided free on-site training in use of the equipment. Novacor did not contribute to study design, study conduct, report preparation or submission.

The allopurinol carotid artery intima media thickness study was competitively funded by a grant from The Stroke Association, UK (TSA 2007/10) and supported by the Scottish Stroke Research Network. The funder did not contribute to study design, study conduct, report preparation or submission.

Following the design of the statistical analysis plan, the blinded statistical analyses relating to the Allopurinol carotid artery intima-media thickness study were performed by Dr Heather Murray and Dr Alex McConnachie, Robertson Centre for Biostatistics, University of Glasgow. The enzyme linked immunosorbent assays were performed by Mr David Hughes. All other work was performed by myself. The writing of this thesis was entirely my own work.

Related Publications

Chapter 3

Higgins P, MacFarlane P, Dawson J, Langhorne P, McInnes GT, Lees KR. Noninvasive cardiac monitoring to detect atrial fibrillation after ischaemic stroke: a randomised controlled trial. *Stroke* 2013; 44; 2525-2531 doi: 10.1161/STROKEAHA.113.001927

Higgins P, Dawson J, MacFarlane P, McArthur K, Langhorne P, Lees KR. Predictive value of newly detected atrial fibrillation paroxysms in acute ischemic stroke patients, for AF after 90 days. *Stroke* 2014 (In Press)

Chapter 4

Higgins P, Dawson J, Walters M. The potential for xanthine oxidase inhibition in the prevention and treatment of cardiovascular and cerebrovascular disease. *Psychiatric and Cardiovascular Neurology* 2009; 2009: 282059 doi: 10.1155/2009/282059 (Epub 2009 Nov 4)

Higgins P, Ferguson LD, Walters MR. Xanthine oxidase inhibition for the treatment of stroke disease: A novel therapeutic approach. *Expert reviews in cardiovascular therapeutics* 2011; 9(4); 399-401 doi: 10.1586/erc.11.29

Chapter 5

Higgins P, Dawson J, Lees KR, McArthur K, Quinn TJ, Walters MR. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. *Cardiovascular Therapeutics* 2012; 30(4); 217-226 doi: 10.1111/j.1755-5922.2011.00277.x.

Chapter 7

Higgins P, Walters MR, Murray HM, McArthur K, McConnachie A, Lees KR, Dawson J. Allopurinol reduces brachial and central blood pressure, and carotid intima-media thickness progression after ischaemic stroke and transient ischaemic attack: A randomised controlled trial. *Heart* 2014 (In Press)

Presentations to Learned Societies

Chapter 3, Study 1

“Evaluation of electrocardiographic monitoring strategy to identify atrial fibrillation in patients with acute ischaemic stroke or TIA”. Platform presentation at the European Stroke Conference (Lisbon, Portugal), 2012. Abstract published in *Cerebrovascular Diseases*, 2012: 33(suppl 2);2-2

Chapter 3, Study 2

“Predictive value in acute ischaemic stroke patients of newly detected atrial fibrillation paroxysms, for the presence of atrial fibrillation after 90 days”. Accepted for E-Poster presentation at the European Stroke Conference (Nice, France), 2014.

Chapter 5

“Xanthine oxidase inhibition for the treatment of cardiovascular disease: A systematic review and meta-analysis”. Platform presentation at the 16th World Congress of Basic & Clinical Pharmacology (Copenhagen, Denmark), 2010. Abstract published in *Basic & Clinical Pharmacology*, 2010: 107(suppl 1); 148.

“Xanthine oxidase inhibition for the treatment of cardiovascular and cerebrovascular disease: A systematic review and meta-analysis”. Poster presentation at the European Stroke Conference (Barcelona, Spain), 2010. Abstract published in *Cerebrovascular Diseases*, 2010: 29 (suppl 2);321.

Chapter 7

“Allopurinol reduces carotid artery CIMT progression and central blood pressure in ischaemic stroke survivors”. E-Poster presentation at the European Stroke Conference (London, UK), 2013. Abstract published in *Cerebrovascular Diseases*, 2013: 35(suppl 3);1-2.

Abbreviations

ACE-I: Angiotensin converting enzyme inhibitor
 ACS: Acute coronary syndrome
 ACTIVE-W: Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events study
 ADP: Adenosine diphosphate
 AF: Atrial fibrillation
 AFFIRM: Atrial Fibrillation Follow-Up Investigation of Rhythm Management
 AG: Augmentation
 AHA: American Heart Association
 AI: Augmentation index
 AI@HR75: Augmentation index standardised for a heart rate of 75 beats per minute
 ALT: Alanine transaminase
 AM: Additional monitoring
 AMP: Adenosine monophosphate
 ANCOVA: Analysis of covariance
 AOI: Area of interest
 AOPP: Advanced oxidation protein products
 ARB: Angiotensin receptor blocker
 ARIC: Atherosclerosis Risk in Communities Study
 ARISTOTLE: Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation study
 ASA: Aspirin
 ASSERT: ASymptomatic atrial fibrillation and Stroke Evaluation in pacemaker patients and the atrial fibrillation Reduction atrial pacing Trial
 AST: Aspartate transaminase
 AT: Atrial tachycardia
 ATP: Adenosine triphosphate
 AVAIL: The Adherence eValuation After Ischemic Stroke Longitudinal registry
 AVERROES: The Apixaban Versus Acetylsalicylic Acid to Prevent Stroke in Atrial Fibrillation Patients Who Have Failed or Are Unsuitable for Vitamin K Antagonist Treatment study
 Ba-PWV: Brachial artery pulse wave velocity
 BAFTA: Birmingham Atrial Fibrillation Treatment of the Aged study
 BP: Blood pressure
 BMI: Body mass index
 BNP: Brain natriuretic peptide
 BP-PWA: Blood pressure pulse wave analysis
 BP-PWV: Blood pressure pulse wave velocity
 CAC: Coronary artery calcium
 CAD: Coronary artery disease
 CAPRIE: Clopidogrel versus aspirin in patients at risk of ischaemic event study
 CAPS: Coronary Atherosclerosis Prevention Study
 CAST: Chinese Acute Stroke Trial
 CCA: Common carotid artery
 CCB: Calcium channel blocker
 CE: Cardioembolism
 CEA: Carotid endarterectomy
 CEM: Continuous electrocardiological monitoring

CHARISMA: Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance trial

CHF: Chronic heart failure

CI: Confidence interval

CIMT: Carotid intima media thickness

CLOTS: Clots in Legs Or sTockings after Stroke

CONSORT: Consolidated Standards of Reporting Trials

COPD: Chronic obstructive pulmonary disease

CRF: Case report form

CRP: C reactive protein

CT: Computed tomography

CVM: Cardiovascular mortality

CVRF: Cardiovascular risk factor

DC: Direct current

DECIMAL: Early decompressive craniectomy in malignant middle cerebral artery infarction trial

DESTINY: Decompressive Surgery for the Treatment of Malignant Infarction of the Middle Cerebral Artery study

DICOM: Digital Imaging and Communications in Medicine

DM: Diabetes mellitus

DNA: Deoxyribonucleic acid

DVT: Deep vein thrombosis

EAFIT: European Atrial Fibrillation Trial

ECASS-3: European Cooperative Acute Stroke Study 3

ECG: Electrocardiograph

ECST: European Carotid Surgery Trial

ELSA: European Lacidipine Study on Atherosclerosis

eNOS: Endothelial nitric oxide synthase

ENOS: Efficacy of Nitric Oxide in Stroke trial

ESO: European Stroke Organisation

ESPRIT: European/Australasian Stroke Prevention in Reversible Ischaemia Trial

ESPS: European Stroke Prevention Study

EXPRESS: Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke study

FBF: Forearm blood flow

FMD: Flow mediated dilatation

FRAP: Ferric reducing ability of plasma

GIST-UK: UK Glucose Insulin in Stroke Trial

GSH: Glutathione, reduced

GSSG: Glutathione disulfide, oxidised

HAEST: Heparin in Acute Embolic Stroke Trial

HAMLET: Hemicraniectomy after middle cerebral artery infarction with life-threatening Edema trial

HbA1C: Glycosylated haemoglobin

HC: Hypercholesterolaemia

Hg: Mercury

HM: Heather Murray

HR: Heart rate

HT: Hypertension

HU: Hyperuricaemia

IA: Intra-arterial

ICA: Internal carotid artery

ICAM: Intercellular Adhesion Molecule

ICD: Intra-cardiac defibrillator
 ICH: Intra-cranial haemorrhage
 i.e.: that is
 IHD: Ischaemic heart disease
 IL: Interleukin
 IMP: Inosine monophosphate
 IMS: Interventional Management of Stroke
 IRAS: Insulin Resistance Atherosclerosis Study
 ISRCTN: International Standard Randomised Controlled Trial Number Register
 IST: International stroke trial
 IV: Intravenous
 KS: Karen Shields
 L: Litre
 LAA: Large artery atherosclerosis
 LACS: Lacunar anterior circulation stroke
 LDL: Low density lipoprotein
 LIFE: Losartan Intervention For Endpoint Reduction in Hypertension study
 LMWH: Low molecular weight heparin
 L-NMMA: L- N-monomethyl arginine
 LVEF: Left ventricular ejection fraction
 LVH: Left ventricular hypertrophy
 MATCH: Management of ATtherothrombosis with Clopidogrel in High-risk patients study
 MDA: Malondialdehyde
 MESA: Multi-Ethnic Study of Atherosclerosis
 Mg: Milligram
 MHRA: Medicines and Healthcare products Regulatory Agency
 MI: Myocardial infarction
 ml: Millilitre
 mm: Millimetre
 μ mol: Micromol
 MOST: Mode Selection Trial
 MRI: Magnetic resonance imaging
 MR-RESCUE: Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy study
 m/s: Metres per second
 n: Number
 NAD: Nicotinamide adenine dinucleotide
 NADH: NAD reduced
 NADP: Nicotinamide adenine dinucleotide phosphate
 NADPH: NADP reduced
 NASCET: North American Symptomatic Carotid Endarterectomy Trial
 NHNESIII: The Third National Health and Nutrition Examination Survey
 NHS: National Health Service
 NICE: National Institute for Health and Care Excellence NIHS: National Institutes of Health Stroke Scale
 NINDS: National Institute of Neurological Disorders and Stroke
 nmol: Nanomol
 NNT: Number needed to treat
 NO: Nitric oxide
 NOAC: Novel oral anticoagulant
 NPV: Negative predictive value
 OAC: Oral anticoagulant

OCSP: Oxford community stroke project
 od: Once daily
 OPT-CHF: The Efficacy and Safety Study of Oxypurinol Added to Standard Therapy in Patients With New York Heart Association Class III-IV Congestive Heart Failure
 OR: Odds ratio
 OXVASC: Oxford Vascular Study
 PACS: Partial anterior circulation stroke
 PAF: Paroxysmal atrial fibrillation
 PAT-RHI: Peripheral arterial tonometry – reactive hyperaemia index
 PCOS: Polycystic ovary syndrome
 PERFORM: Prevention of cerebrovascular and cardiovascular Events of ischaemic origin with teRutroban in patients with a history of ischaemic stroke or tRansient ischaemic attack study
 PFO: Patent foramen ovale
 PICSS: PFO in Cryptogenic Stroke Study
 POCS: Posterior circulation stroke
 PP: Pulse pressure
 PPAR: Peroxisome proliferator-activated receptor
 PPM: Permanent pacemaker
 PPV: Positive predictive value
 PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
 PROactive: PROspective pioglitAzone Clinical Trial
 PROBE: Prospective randomised open label blinded endpoint study
 PROfESS: Prevention Regimen for Effectively Avoiding Second Strokes
 PROGRESS: Perindopril Protection Against Recurrent Stroke Study
 PROTECT-AF: Watchman Left Atrial Appendage System for Embolic Protection in Patients With Atrial Fibrillation study
 PWM: Peter MacFarlane
 QALY: Quality adjusted life year
 QUORUM: Quality of Reporting of Meta-analyses
 RAA: Renin - Angiotensin - Aldosterone
 RCB: Robertson centre for Biostatistics, University of Glasgow
 RCT: Randomised controlled trial
 R&D: Research & Development
 RE-LY: Randomized Evaluation of Long-term Anticoagulation Therapy study
 ROCKET-AF: Rivaroxaban Once Daily Oral Direct Factor Xa Inhibition Compared with Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation study
 RNA: Ribonucleic acid
 ROS: Reactive oxygen species
 RR: Relative risk
 rt-PA: Recombinant tissue plasminogen activator
 SD: Standard deviation
 SE: Systemic embolism
 SEM: Standard error of the mean
 SIGN: Scottish Intercollegiate Guidelines Network
 SITS-ISTR: Safe Implementation of Treatments in Stroke International Stroke Thrombolysis Register
 SITS-MOST: Safe Implementation of Treatments in Stroke Monitoring Study
 SP: Standard practice
 SP-AM: Standard practice with additional monitoring
 SPARCL: Stroke Prevention by Aggressive Reduction in Cholesterol Levels
 SUA: Serum uric acid
 SVD: Small vessel disease

TACS: Total anterior circulation stroke
TAS: Total antioxidant status
TE: Thromboembolic
TED: Thromboembolic disease
TIA: Transient ischaemic attack
TOAST: Trial of Org 10172 in Acute Stroke Treatment
Tr: Time to reflection
TRENDS: A Prospective Study of the Clinical Significance of Atrial Arrhythmias Detected by Implanted Device Diagnostics
UA: Uric acid
UFH: Unfractionated heparin
UK: United Kingdom
UPAW: Unavailability of patient to attend within protocol allocated window
USA: United States of America
VACS: Veterans Affairs Cooperative Study
VA-SPINAF: Veterans Administration Stroke Prevention in Atrial Fibrillation study
VEGF: Vascular endothelial growth factor
VISTA: Virtual International Stroke Trials Archive
VKA: Vitamin K antagonist
VTE: Venous thromboembolism
WARSS: Warfarin-Aspirin Recurrent Stroke Study
XO: Xanthine oxidase
XOI: Xanthine oxidase inhibition

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Chapter 1

The need for optimised secondary preventative strategies in reducing the burden of cerebrovascular disease

1.1. The burden of cerebrovascular disease

Stroke is defined by the World Health Organisation as a clinical syndrome of “rapidly developing clinical signs of focal and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin” ¹ Approximately 80% of cases are accounted for by ischaemic pathology. Haemorrhagic pathology, either intracerebral haemorrhage (10%) or subarachnoid haemorrhage (5%), accounts for the majority of the remainder of cases ². Stroke has risen to hold status as the 2nd leading single cause of adult mortality globally ³ and is a leading cause of disability in the adult population ^{4, 5}.

1.1.1. Incidence

Stroke is principally a disease of the elderly population, with incidence rising exponentially with age ⁶. Recent decades have witnessed a 42% decline in the age-specific incidence of stroke in developed countries ⁷. General Practice Research Database data suggests that the incidence is continuing to fall in the United Kingdom (UK), with a 30% reduction reported between 1999 and 2008 ⁸. These findings have been attributed in large part to the emergence of robustly implemented blood pressure (BP) screening and cardiovascular risk factor (CVRf) management programmes ^{8, 9}.

Whilst age-specific incidence of stroke may have fallen, the elderly population has increased in size in recent years and is anticipated to continue to do so ¹⁰. As the incidence of stroke is highest in the elderly population, the absolute number of stroke hospital admissions has increased in recent years ¹¹⁻¹³.

Recent years have also witnessed a rise in stroke incidence in younger age groups. In a combined analysis of the Greater Cincinnati & Northern Kentucky populations, the age at first stroke fell by two years between 1993-94 and 1999 – 2005 and events in the population aged under 55 years comprised almost one fifth of all cases in the latter time period ¹⁴.

Geographical variation in stroke incidence was observed 20 years ago ¹⁵, with the highest rates observed in the developed world. Subsequently, the effects of globalisation have given rise to increased age-specific incidence of stroke in developing countries in recent decades, with approximately 70% of global stroke deaths now occurring in developing countries ^{7, 16}.

The incidence of stroke remains high in the UK ¹³. In England, stroke incidence was reported as 178 and 139, for males and females respectively, per 100,000 in 2007 ¹⁷. There is regional variation within the UK, with higher incidence in Scotland at 208 and 159 cases per 100,000 ¹⁸.

Based on the incidence reported in the Oxford Vascular Study (OXVASC), the number of first instances of ischaemic stroke in the UK was estimated at 98,000 annually, with an additional 46,000 incident cases of TIA ^{12, 17} 8,500 haemorrhagic strokes and 5000 subarachnoid haemorrhages were also expected. These estimates were considered likely to be conservative, based on the affluent status of the OXVASC population and higher regional incidence elsewhere. Based on the combined regional stroke incidence, an estimated 150,000 incident strokes occur annually in the UK ¹³. In addition to incident cerebrovascular events, a large number of patients experience recurrent events each year, comprising one quarter of all annual strokes ^{18, 19}.

Due to the simultaneous expansion of the elderly population, increasing incidence in younger age groups and in the developing world, the global burden of stroke is anticipated to rise in the coming decades. By 2030, 7.8 million stroke-attributable deaths are anticipated globally compared with 5.7 million in 2005 and the number of disability-adjusted life years lost to stroke is expected increase from 50.8 million to 60.9 million ¹⁶.

1.1.2. Mortality

Stroke has risen to hold status as the 2nd leading single cause of adult mortality globally ³, reflecting high incidence in the developed world, increasing incidence in the developing world and a persistently high case fatality rate ⁷.

Despite improved availability of stroke unit care and development of services implementing treatment with thrombolysis, case fatality rates (recorded at 60 days) remain high, at 18.7% and 25.2% of males and females, respectively in Scotland¹³. In the Oxford community stroke project (OCSP) population, 1-year case fatality was 60% in the most severe clinical category of stroke and varied between 11 – 19% for other clinical categories²⁰.

Thus, stroke related mortality remains high, with age-standardised death rates of 51 males and 48 for females, respectively, per 100,000 in the UK in 2007¹². Stroke accounts for around 50,000 deaths in the UK annually^{12, 13}, accounting for 9% of adult deaths and representing the second leading single cause of adult death in the UK¹².

Mortality varies widely with geographical region. Rates in Scotland are higher than for the UK average, at 65 (males) and 58 (females) deaths per 100,000 population¹². Mortality rates are broadly similar between most European Union nations but are markedly higher in eastern European and non-European developing countries²¹. Up to 10-fold differences in mortality have been observed between developed and developing countries²². Death rates appear related to socioeconomic status, including within developed countries such as the UK, where manual occupation class mortality is approximately twice that observed within the non-manual classes¹².

1.1.3. Morbidity & societal burden

Approximately 2 – 3% of the UK population is affected by stroke^{12, 13}. Prevalence increases with age. In 2006, 13% of males and 9% of females over 75 years had suffered a previous stroke¹². With improved survival rates and an expanding elderly population, the prevalence of stroke survivors is anticipated to rise.

Approximately half of stroke survivors become dependent on others to some degree⁴. Likelihood of a good functional recovery is related to the clinical severity at the time of presentation with stroke. In the OCSP, depending on the clinical nature of the stroke deficit, 19 – 36% of patients surviving at 1-year did so with severe levels of disability and dependency²⁰. UK data, from the era of modern management, suggest that approximately

one fifth of patients suffer severe residual disability associated with high levels of dependency²³. Consequently, stroke represents a leading cause of disability in the adult population^{4, 5} and requirement for institutional care²⁴.

Beyond the human cost of stroke to patients and their families, stroke carries a huge societal financial burden. In the UK, stroke accounts for 1 – 2% of all hospital admissions¹³. With lengthy admission durations (mean 20 days)²³, healthcare related costs are disproportionately high for stroke patients, accounting for an estimated 1% - 5% of annual healthcare budgets^{13, 25}.

Most patients who suffer stroke survive their first episode¹³. Many do so with residual disability^{4, 23} and all remain at risk of recurrent stroke. Accordingly, the financial burden of stroke comprises not only the healthcare costs during hospital based acute and rehabilitation management but also the subsequent cost of institutional and community based social care for patients with residual disability. Consequently, the financial cost of stroke is huge, estimated at between £4 - 7 billion per year in the UK alone^{13, 24, 26}.

1.2. Management of acute stroke

1.2.1. Management principles

Patients presenting with acute ischaemic stroke are at risk of sustaining fatal or disabling neurological injury. All patients remain at risk of a recurrent and often more severe stroke. Survivors are also at risk of early stroke-related medical complications, particularly infection, venous thromboembolism and cardiac complications^{27, 28}.

Accordingly, management of patients with acute stroke is based on attempting to limit the extent of initial neurological injury and providing supportive physiological monitoring and therapy to reduce the risk of complications²⁸. Management seamlessly proceeds to assessment for underlying aetiological mechanisms and initiation of secondary preventative measures²⁹. Rehabilitation is commenced at the earliest opportunity to optimise functional recovery³⁰.

1.2.2. The ischaemic penumbra

Following arterial occlusion, cerebral blood flow and perfusion pressure each fall within the territory supplied by the affected vessel ³¹. Metabolic demand remains constant and cellular ischaemia ensues, progressing over time to irreversible infarction ³². Typically, a central core of tissue infarction develops at the site of arterial occlusion, which subsequently expands to encompass the surrounding volume of ischaemic but potentially viable tissue, referred to as the penumbra ³³. However, avoidance of progression to infarction is dependent on arterial recanalisation and restoration of perfusion ³⁴. Penumbral tissue may be salvageable for a period of several hours, providing a window of opportunity for either spontaneous or therapeutic recanalisation ³⁵.

Therapeutic interventions to improve outcome in acute stroke have been focused on attempts to achieve recanalisation of the occluded artery and in turn restoration of cerebral perfusion. In addition, studies have examined supportive manipulation of deranged physiological variables which appear to influence clinical outcome and putative neuroprotectant strategies which may delay or prevent tissue infarction ²⁸.

1.2.3. Reperfusion strategies

1.2.3.1. Intra-venous thrombolytic therapy

Three large randomised controlled trials (RCTs) have each demonstrated benefit of intravenous (IV) thrombolytic therapy in reducing death and significant disability. The NINDS study demonstrated benefit of treatment with Alteplase, initiated within three hours of symptom onset, with an odds ratio (OR) of a favourable 3-month outcome of 1.7 (95% CI, 1.2 – 2.6) compared with placebo ³⁶. The ECASS-3 study demonstrated benefit of Alteplase in an extended time window, between 3 to 4.5 hours from symptom onset, with an OR for a favourable 3-month outcome of 1.34 (95% CI, 1.02 – 1.76), compared with placebo ³⁷. Meta-analysis of studies examining intravenous thrombolysis concluded significant benefit existed with treatment commenced up to 4.5 hours from symptom onset ³⁸. Benefit of treatment declines with time from symptom onset; compared with placebo, the OR for a favourable 3-

month outcome for those treated with 90 minutes of symptom onset is 2.55 but falls to 1.34 (95% CI, 1.06 – 1.68) for those treated between 180 and 270 minutes. Phase IV studies have confirmed similar benefit in routine clinical practice to that reported in the RCTs^{39, 40}. Such studies have also indicated significant benefit amongst groups who were excluded from the clinical trial populations, including more elderly patients⁴¹, diabetics and those with history of prior stroke⁴². The results of the IST-3 study, an open label RCT comparing recombinant tissue plasminogen activator (rt-PA) with no treatment were published in 2012. These results confirmed the benefit observed in observational data amongst more elderly patients⁴³. Clinical guidelines recommend treatment with (IV) rt-PA for all eligible patients²⁸.

Current research regarding use of IV thrombolysis is focused on identifying patients in whom treatment may potentially be advantageous but who present either with an unclear time of symptom onset or beyond the conventional 4.5 hour treatment window. These efforts have been focussed on identification of ischaemic core and viable penumbra, through computed tomography (CT) perfusion or magnetic resonance imaging (MRI) diffusion and perfusion weighted mismatch imaging techniques³⁴. Clear benefit of this approach in patient selection is yet to be shown⁴⁴.

1.2.3.2. Intra-arterial neuroradiological procedures

Whilst IV thrombolytic therapy services may be readily implemented in most hospital settings, many patients are ineligible for treatment, having either a contra-indication to systemic thrombolytic therapy, or presenting outwith the treatment time window. Many patients receiving IV treatment still experience an unfavourable outcome, in part reflecting vessel recanalisation being achieved in only 30 – 40% of treated patients and fewer in large artery occlusions⁴⁵. For these reasons, establishing reperfusion through interventional neuro-radiological procedures has been explored.

Intra-arterial (IA) thrombolysis up to six hours after symptom onset has been studied in relation to middle cerebral artery occlusion in several small studies⁴⁶. In meta-analysis, IA thrombolysis was associated with favourable odds of a good clinical outcome compared with non-active control (OR 2.05; 95%CI, 1.33 – 3.14). Trial numbers are small however and such

treatment is less readily available than intravenous therapy. In addition, there are concerns regarding the risk of intracerebral haemorrhage as a complication of intra-arterial therapy. In the PROACT II study, symptomatic haemorrhagic transformation was observed in 10% of treated patients ⁴⁷. In patients with basilar occlusion, randomised controlled studies are lacking but the available observational data identified no difference in outcomes between intravenous and intra-arterial thrombolysis ⁴⁸.

Mechanical thrombectomy has been examined in numerous retrospective & prospective observational studies and case series reports. Arterial recanalisation rates with various thrombectomy techniques and devices vary, are typically higher than those quoted with IV therapy but are also associated with higher rates of symptomatic intracranial haemorrhage (ICH) ⁴⁹. Randomised data have until recently been lacking. Results from the Synthesis Expansion study, which compared endovascular treatment with IV rt-PA, found no difference in rates of disability between the two treatment groups 3 months (OR 0.71 (95% CI, 0.44 - 1.14) ⁵⁰. However, only around one third of patients underwent mechanical thrombectomy, with the remainder receiving IA rt-PA alone. The IMS III study which evaluated the combination of IV and IA (including mechanical thrombectomy) treatment, compared with IV treatment alone, reported discontinuation of enrolment after crossing a futility boundary ⁵¹. The MR-RESCUE study compared IV rt-PA with mechanical thrombectomy and also reported no difference in 3 month outcomes between treatment groups ⁵². At present the use of endovascular therapy is recommended in the context of clinical trials.

1.2.4. Antiplatelet therapy

In the first International Stroke Trial (IST), amongst patients with suspected ischaemic stroke, early institution (within 48 hours of ictus) of Aspirin (ASA) 300mg once daily for 14 days was found to reduce death or dependency at 6 month follow up compared with control, with 14 fewer dead or dependent patients per 1000 treated (2p < 0.05, after adjustment for baseline prognosis). It appears likely that the observed treatment effect was attributable to a reduction in early ischaemic stroke recurrence ^{28, 53}. The placebo controlled CAST study reported similar benefits of early ASA therapy, with a 14% reduction in odds of death at 28

days ($2p = 0.04$), again driven by a significant reduction in recurrent ischaemic stroke events⁵⁴. The benefit of ASA in ischaemic stroke may have been underestimated in these studies, which randomised one third of patients prior to CT imaging to exclude haemorrhagic pathology. Guidelines recommend institution of antiplatelet therapy within 24 hours in ischaemic stroke patients, following exclusion of haemorrhage with brain imaging. Treatment with Aspirin is recommended in the initial 14 days on the basis that this agent was studied in the IST and until recently, Clopidogrel lacked an evidence base supporting use in the days immediately following symptom onset. A recent study, conducted in a Chinese population, identified reduced stroke recurrence with combination therapy of Aspirin and Clopidogrel compared with Aspirin monotherapy (HR 0.68, 95% CI; 0.57 to 0.81)⁵⁵.

1.2.5. Anticoagulant therapy

Anticoagulants may putatively hold benefit through prevention of neurological deterioration or enhanced neurological recovery. Prevention of early recurrent embolisation may also improve patient outcomes, particularly in patient subgroups at increased risk for this.

The largest trial to examine the role of early anticoagulant therapy was the IST⁵³, which randomised 19,435 patients with suspected ischaemic stroke to receive fixed dose subcutaneous unfractionated heparin (UFH) or not, within 48 hours of symptom onset. Despite a significant reduction in ischaemic stroke events in the UFH group (2.9% versus 3.8%, $2p < 0.01$), the study failed to demonstrate overall benefit due to an excess in ICH and extracranial bleeding with (UFH) treatment. Similar findings were observed in a subgroup of patients with atrial fibrillation (AF)⁵⁶. Interpretation of these results is problematic due to methodological flaws in the IST, including lack of exclusion of ICH with CT imaging prior to administration of randomised therapy, concomitant administration of high dose ASA in 50% of the UFH group (the authors reported no significant interaction but without presentation of subgroup analysis), lack of therapeutic monitoring and lack of subgroup reporting based on stratification according to size of cerebral infarction on CT imaging.

Several smaller studies have also examined early treatment with a variety of anticoagulants, including UFH, low molecular weight heparin (LMWH) and other heparinoids. The TOAST

study was a placebo controlled trial of the heparinoid Danaparoid (Org10172), commenced intravenously within 24 hours of ischaemic stroke onset and continued for 7 days⁵⁷. In this relatively mild stroke population, odds of a favourable 3-month outcome with Danaparoid were similar to with placebo (OR 1.13, 95% CI 0.57 – 2.24). Recurrent ischaemic stroke events were similar between groups at 7 days but patients treated with Danaparoid experienced higher rates of ICH and extra-cranial bleeding.

The HAEST study compared subcutaneous Dalteparin with ASA, commenced within 30 hours of acute ischaemic stroke, in patients with AF⁵⁸. The study population had relatively severe stroke, with 65.5% of patients experiencing an outcome of either death or dependency at 90-day follow up. Additionally, baseline systolic BP was significantly higher in the LMWH group. No difference in functional outcome was observed between treatment groups at 90-days. Odds of recurrent ischaemic stroke were similar between treatment groups (OR 1.13, 95% CI 0.57 – 2.24), while Dalteparin was associated with an increase in a composite measure of early recurrent ischaemic stroke, stroke progression, symptomatic ICH and death (OR 1.60, 95% CI 1.01 – 2.54).

Meta-analysis of trial data examining treatment with anticoagulants in acute stroke of cardioembolic cause suggests that early ischaemic stroke recurrence may be reduced compared with non-anticoagulant treatment strategies (OR 0.69, 95% CI 0.44 – 1.06). However, symptomatic ICH is increased (OR 2.89, 95% CI 1.19 – 7.01) and no difference is observed in relation to death or disability at subsequent follow up (OR 1.01, 95% CI 0.82 – 1.24)⁵⁹. Accordingly, guidelines do not routinely recommend use of anticoagulation in acute ischaemic stroke of either presumed arterial or cardioembolic aetiology²⁸, though limitations of trial design mean that potential benefit in particular subgroups has not been excluded. Evidence regarding early anticoagulation in TIA patients is lacking.

1.2.6. Control of physiological variables

1.2.6.1. Blood pressure

Elevated BP in acute ischaemic stroke patients is associated with both increased mortality and poor functional outcome ⁶⁰. However, the relationship between systemic BP and stroke outcome appears to be complex. A non-linear relationship, following a U-shaped curve, with poorer outcomes reported amongst patients with both the highest and lowest admission BPs was observed in the IST ⁶¹. Conversely, a more linear relationship between clinical outcome and average BP over the days following stroke has been reported in other study populations, with a less apparent relationship reported with admission BP ⁶². Elevated BP may confer poorer outcome through predisposing to haemorrhagic transformation, (including in the context of thrombolytic therapy), increased cerebral oedema and other end organ damage ²⁸. Conversely, elevated systemic BP may preserve cerebral blood flow, which is linearly related to systemic BP in the context of impaired cerebral autoregulation during acute stroke ⁶³. The optimal systemic BP remains undetermined and may vary according to stroke subtype and individual patient factors.

Several studies have examined the role of acute BP reduction in the setting of acute ischaemic stroke. None have demonstrated definite benefit with treatment, though lack of statistical power has limited interpretation. The results of the large ENOS study, examining early BP lowering with nitrate based treatment are anticipated in the near future. Current guidelines make only limited recommendations regarding manipulation of BP in acute stroke, reflecting the underpowered and equivocal nature of the available data ²⁸.

1.2.6.2. Serum glucose

Hyperglycaemia is highly prevalent amongst patients presenting with acute stroke, including amongst non-diabetic patients ⁶⁴. This in part reflects a group with hitherto undiagnosed, occult diabetes mellitus (DM) ⁶⁵. However, hyperglycaemia also appears to represent a component of the stress response to the acute physiological insult ⁶⁶. Larger infarct burden on brain imaging is associated with hyperglycaemia ⁶⁷. The level of admission and subsequent in-patient hyperglycaemia is associated with clinical outcome, with higher blood glucose levels predicting adverse prognosis ^{66, 68}. In addition, patients with hyperglycaemia

treated with thrombolytic therapy are more likely to experience haemorrhagic transformation⁶⁹.

Randomised evidence assessing the treatment of hyperglycaemia in acute stroke is limited. The largest study was the GIST-UK study⁷⁰, which terminated prematurely due to slow patient enrolment. No significant clinical effect of treatment with a GKI regime was observed, although the reduction in serum glucose was modest and active treatment was associated with a moderate reduction in BP. Variable rate insulin infusions^{71, 72} achieve lower blood sugar levels but at the expense of increased hypoglycaemic episodes. Clinical outcomes in relation to treatment have not been assessed.

1.2.6.3. Body temperature

Elevated body temperature in patients with acute stroke is associated with adverse outcome. Whilst potentially attributable to central mechanisms, pyrexia may also indicate infection or thrombotic phenomena complicating the post stroke period. Raised temperature is conducive to increased metabolism, which in the context of cerebral ischaemia may accelerate tissue infarction. Several small studies have examined the potential benefit of modest induced hypothermia as a neuroprotective strategy during acute ischaemic stroke⁷³. Results have been inconclusive but larger studies are now enrolling patients.

1.2.7. Neuroprotection

In the context of cerebral ischaemia, a number of cellular metabolic pathways have been considered potential therapeutic targets for manipulation, with a view to delaying the progression to cellular death, until tissue reperfusion, by whatever means, may occur. Potential targets have included glutamate, calcium flux, cellular proteases, apoptosis, oxygen free radicals and inflammatory pathways²⁸. A large number of clinical trials have been conducted in acute stroke which have examined the therapeutic manipulation of these mechanisms of injury⁷⁴. To date none has demonstrated efficacy in improving clinical outcome following stroke and the use of neuroprotective agents is not recommended.

1.2.8. Surgical strategies

Malignant middle cerebral artery syndrome is a complication of proximal middle cerebral or terminal internal carotid artery occlusion, which may develop approximately 48 hours following occlusion. Extensive infarction-related cerebral oedema results in raised intracranial pressure, mass effect and tentorial herniation²⁸. Mortality is high and survivors are invariably severely disabled. The data from the HAMLET, DESTINY and DECIMAL trials, which each compared emergent decompressive hemicraniectomy with medical management alone, were combined in a meta-analysis which demonstrated a highly significant reduction in mortality^{75, 76}. The number of patients needed to treat to prevent one death was only two, though survivors were frequently severely disabled at follow up. These studies were conducted in patients below 65 years in age. A recent trial has demonstrated a significant reduction in mortality in patients up to 75 years in age, though again with the majority of survivors experiencing severe levels of residual disability⁷⁷.

1.2.9. Systems of care

Prompt admission to a specialist acute stroke unit, with access to medical, nursing, physiotherapy, occupational therapy, speech and language therapy staff with specialist experience in the management of stroke patients, has been shown to improve patient outcomes. These include improved functional recovery and fewer patients experiencing an outcome of either institutionalisation or death⁷⁸. The contribution of the individual components of stroke unit care to the observed treatment benefit has not been elucidated further; a “black box” treatment effect of stroke unit care has consequently been described. Irrespective of the mechanism of benefit, prompt admission to a specialist stroke unit is recommended for all stroke patients^{27-29, 79}.

1.2.10. Prophylaxis of post-stroke venous thromboembolic disease

Venous thromboembolic (VTE) disease is a common complication following stroke, particularly amongst more severely affected patients who are rendered immobile. Low dose

anticoagulation, with either UFH or LMWH, reduces the frequency of deep vein thrombosis (DVT) following stroke. However, routine use has not been recommended due to concerns over treatment predisposing to haemorrhagic transformation of acute infarct⁸⁰.

The CLOTS and CLOTS-2 trial investigators concluded no overall benefit to support the use of either below or above knee length graduated compression TED stockings. Despite a reduced incidence of DVT, a small excess in skin ulceration associated with stocking use was observed⁸⁰. In the CLOTS-3 trial, use of intermittent pneumatic compression was found to reduce the incidence of proximal DVT in patients following stroke, with a number needed to treat (NNT) to prevent one DVT of 28 patients⁸¹. Proximal DVT was evident on ultrasound imaging in 12.1% of control patients but symptomatically so in only one third, suggesting there may be a role for routine DVT screening following stroke.

1.2.11. Clinical outcomes following stroke

Admission to a specialist acute stroke unit is universally applicable to all stroke patients. Despite efforts to establish healthcare models by which to deliver reperfusion treatment, this is rarely achieved in more than 10% of ischaemic stroke patients^{23, 82, 83}, primarily reflecting the constraint of time from ictus to the delivery of both safe and effective treatment. The NNT for early initiation of ASA therapy is relatively high. Other therapeutic strategies are either limited to relatively small subgroups of patients or lack a robust evidence base. Despite improved outcomes with treatment with rt-PA and management in specialist stroke centres, approximately half of patients in the placebo arm of contemporary studies still experienced an unfavourable outcome (death or dependency) at 90 days³⁸.

Thus, a reduction in the burden of stroke disease is dependent on the identification and implementation of effective preventative strategies. This is a particularly important aspect of management given the high risk of recurrent stroke following index stroke or TIA.

1.3. The risk of stroke recurrence following ischaemic stroke & TIA

1.3.1. Clinical and aetiological classification systems for ischaemic stroke

The Oxford classification system categorises patients according to their clinical pattern of presentation as having experienced one of four stroke syndromes (table 1-1) ²⁰. This system provides useful prognostic information regarding likelihood of functional recovery and in relation to risk of stroke recurrence. The TOAST classification system considers the combination of the clinical stroke syndrome, infarct topography on brain imaging, vascular & cardiac imaging, electrocardiological studies and laboratory investigations in defining ischaemic stroke according to one of five aetiological categories: large artery atherosclerosis (LAA); small vessel disease (SVD); cardioembolism (CE); other determined pathology; undetermined pathology ⁸⁴.

Table 1-1: The Oxford clinical stroke classification system

(reproduced from Bamford et al, Lancet 1991)

Prognosis at 1 year	TACS	PACS	LACS	POCS
Dead	60 %	16 %	11 %	19 %
Dependant	36 %	29 %	28 %	19 %
Independent	4 %	55 %	60 %	62 %
TACS, total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar anterior circulation stroke; POCS, posterior circulation stroke.				

The relationship with outcome reflects the link between the clinical syndrome and infarct topography on brain imaging, which in turn is indicative of potential aetiological mechanisms: lacunar syndrome (LACS) events are associated with lacunar infarcts, indicating SVD, whilst

partial and total anterior circulation events (PACS & TACS, respectively) are associated with embolic pattern infarcts, indicative of either a LAA or CE aetiological mechanism⁸⁵. The latter categories appear to be associated with higher risk of recurrence, as discussed in further detail below. In a small minority of cases, other causes including cervical artery dissection, arterial vasculitis, underlying thrombophilia and venous thrombotic disease may each give rise to cerebral ischaemia³².

Historical studies examining the natural history of stroke have utilised diverse classification systems, limiting direct comparisons between populations and time periods. The wider adoption of TOAST provides a basis for direct comparability between study populations and assessment of prognosis and treatment effects within aetiological subgroups⁸⁶. However, patients with undetermined pathology represent a heterogeneous cohort, comprising both those in whom either no aetiological factor is identifiable but also those patients in whom more than one aetiological factor is identified through investigation (following either comprehensive or incomplete investigation). I.e. the presence of both LAA and AF would result in an undetermined aetiological classification. Consequently, assignment of risk and optimal treatment may be more problematic in this group.

1.3.2. Prevalence of co-existent aetiological mechanisms

Conventionally, LAA, SVD and CE have been considered the main causes of ischaemic stroke, accounting for 13.4 – 16.7%, 15.9 – 22.6% and 18.6 – 29.1% of cases respectively⁸⁷. However, as each of these aetiological categories share similar underlying risk factors, combinations (or all) of these potential culprit pathologies are identified to coexist in a substantial proportion of patients with ischaemic stroke & TIA. This is supported by the largest aetiological category being “undetermined”, accounting for 34.7 – 42.2% of cases⁸⁷.

In patients with AF, the CHA2DS2-VASC score predicts the presence of concomitant cerebral artery atherosclerosis⁸⁸. Approximately 25% - 30% of patients found to have AF at the time of ischaemic stroke will also have demonstrable evidence of LAA^{88, 89}. In one consecutive series of patients with acute brain ischaemia, 188 of 300 patients were identified as having a potential cardiac source of embolism⁹⁰. 84.6% of these had competitive aetiologies,

predominantly LAA. In one third of patients with a stroke and AF, the cerebral event may be causally related to a co-existent arterial lesion, rather than AF-related cardioembolism^{91, 92}.

Conversely, in patients with severe carotid stenosis, AF was noted in 13.7% in one recent case series⁹³ and in the NASCET clinical trial population, which included ischaemic stroke & TIA patients with evidence of extracranial internal carotid artery (ICA) atherosclerosis of varying degree, during 5-year average follow up, 20% of recurrent strokes in the territory of the originally symptomatic artery were considered to be unrelated to the carotid stenosis⁹⁴. Thus, the conventional proportional assignment of stroke aetiology may represent an oversimplification.

1.3.3. Risk of recurrence following ischaemic stroke

Approximately 152,000 strokes occur in the UK each year. Approximately 129,000 of these are ischaemic stroke and in addition, 46,000 patients also suffer a first TIA^{9, 12}. All these patients remain at risk of a further, potentially disabling stroke. Indeed, incident strokes represent only around three quarters of all annual stroke events, with the remainder comprising recurrent events^{18, 19}. In patients who experience mild ischaemic stroke or TIA, subsequent events are major & disabling in approximately two thirds of cases^{32, 95}. In addition, these patients are at high risk of subsequent cardiac events and cardiovascular death³². Thus, incident stroke, particularly mild, non-disabling stroke events and TIA, identifies a group of patients at risk of future disabling or fatal stroke and other cardiovascular events.

Stroke recurrence rates reported in studies vary according to the characteristics of the population studied, including concomitant risk factors for stroke, age and secondary preventative measures contemporary to the period of study. Definition of stroke recurrence and duration of follow up also influence reported rates³². There is some heterogeneity in subsequent risk between patients with completed stroke and those with TIA (or minor stroke). In addition, the risk of subsequent events may be stratified further according to concomitant risk factors and the underlying stroke mechanism.

Population studies indicate a high long term risk of recurrence following stroke ⁹⁶. A systematic review & meta-analysis of published hospital and community based stroke registries, estimated the 5- and 10-year cumulative risk of recurrence to be 26.4% (95% CI, 20.1 – 32.8%) and 39% (95% CI 27.2 – 51.2%), respectively ⁹⁷. Although long term cumulative risk of recurrence is high, the highest risk period is during the initial year following an index stroke, with approximately one quarter of the 10-year cumulative recurrent event risk accounted for by events in this period, with 11.1% (95% CI, 9.0 – 13.3%) experiencing recurrent stroke. The first 30-days following the index event appeared to be the highest risk period of all, with 3.1% (95% CI, 1.7 – 4.4%) of patients suffering recurrence.

In this analysis, significant heterogeneity in recurrence rates between studies was noted, reflecting differences in population characteristics and methodology among published studies. Irrespective of this, the risk of stroke recurrence was high across all studies, with estimates ranging between 1.1% - 15% by one year and 14% to 51.3% by 10 years ⁹⁷.

1.3.4. Risk of stroke following TIA

Estimates of stroke risk following TIA also vary according to the population studied ³². However, irrespective of the population context, this group is at increased risk of stroke and other vascular events compared with age & sex matched controls ^{98, 99}. 15 – 30% of patients who suffer a disabling stroke have experienced a prior TIA ¹⁰⁰. In the OCSP, patients with a TIA had a 13-fold excess risk of stroke in the subsequent 12 months and 7-fold excess over the 7 years following TIA ⁹⁸.

Studies indicate that the risk of early and very early stroke recurrence following TIA appears to be higher than in patients with completed stroke. Whilst this may, at least in part, reflect methodological aspects of study design, this has prompted revision of the approach to management of TIA, with the condition now regarded as a medical emergency, potentially heralding disabling or fatal stroke ¹⁰¹.

Amongst patients who presented to Californian hospital emergency departments with suspected TIA, 5.3% of patients re-presented with completed stroke within 48 hours. 10.5%

suffered stroke within 90 days⁹⁵. In the OCSF population, 8.6% (95% CI 4.8 – 12.4%) and 12.0% (95% CI, 7.6 – 16.4%) of patients, respectively, suffered stroke within 7 and 30 days of their index symptoms¹⁰². 15 – 30% of patients who suffer a disabling stroke have experienced a prior TIA and up to 23% of patients who suffer a completed stroke report a TIA in the preceding week¹⁰⁰. Stroke following TIA is disabling in approximately two thirds of patients⁹⁵.

In a systematic review and meta-analysis of studies examining early risk of stroke following TIA¹⁰³, heterogeneity was observed in recurrence rates, which varied between populations and according to the clinical management of TIA. Higher recurrence was observed in populations with delayed, as opposed to expedited, investigation and commencement of secondary preventative measures. In studies examining populations lacking expedited medical care, 2- and 7-day stroke occurrence was 6.7% (95% CI, 3.6 – 9.7%) and 10.4% (95% CI, 8.1 – 12.6%), respectively. Expedited medical assessment, investigation and institution of secondary preventative measures have been shown to reduce the early recurrence following TIA and minor stroke^{103, 104}. Nevertheless, the risk of stroke remains high even in this context.

Recent years have seen the development and validation of risk stratification tools for the assessment of patients following TIA, identifying certain patients to be at particularly increased risk relative to the TIA population as a whole¹⁰⁵. According to the presence of risk factors (age, elevated BP, DM) and clinical features (lateralising motor deficit, speech disturbance, symptom duration), patients may be stratified variously as low, intermediate or high risk of early stroke occurrence following TIA. In validation studies, patients with high risk scores have been reported to have stroke rates of 8.1% within 2 days of TIA¹⁰⁶.

Patients with TIA and ischaemic stroke of mild severity are qualitatively similar in terms of age, sex & prevalence of co-existent vascular diseases & risk factors and accordingly have similar prognosis in terms of risk of subsequent stroke and vascular mortality^{107, 108}. The risk of stroke following ocular TIA (amaurosis fugax), appears to be lower to that following cerebral TIA¹⁰². Patients who survive the initial 12 months following TIA event free remain at elevated risk, with a recurrent stroke rate of approximately 5% per annum^{32, 98}.

1.3.5. Risk of non-stroke events following ischaemic stroke & TIA

Whilst the majority of vascular events arising subsequent to a stroke or TIA comprise a completed stroke, patients also appear to be at high risk of other cardiovascular events, including coronary artery disease (CAD) events and vascular death. In the placebo arm of the SPARCL study, 8.9% of patients suffered a serious non-stroke cardiovascular event during 4.9 years of follow up¹⁰⁹. The risk of non-stroke cardiovascular events, including cardiovascular death is also high (25.1% of patients within 3 months) following TIA⁹⁵.

1.3.6. Risk of recurrence according to aetiological subtype

Following both stroke and TIA, individual risk of subsequent stroke is influenced not only by the timing of intervention with medical investigation and institution of secondary preventative therapies but also by the presence or absence of potentially culprit aetiological & risk factors.

Population studies which characterised patients according to their aetiological subtype provide information regarding the natural history and recurrence risk according to the presence of particular aetiological factors. Data from closely monitored clinical trial populations provide an estimate of recurrence rates in the context of specific and well characterised aetiological subtypes of stroke.

One analysis of population based studies, which retrospectively applied TOAST criteria, suggested risk of early recurrence was greatest in patients with confirmed LAA, followed by patients with either cardioembolic or undetermined aetiology¹¹⁰. Patients with SVD related lacunar strokes appear to be at reduced risk of early recurrence compared with the stroke population as a whole. Conversely, analysis of the Erlangen stroke registry¹¹¹ reported similar risk for LAA and CE related stroke early after the incident event. SVD related stroke was again associated with the lowest rate of recurrence.

It appears that the relative risk (RR) of stroke recurrence according to stroke subtype may vary with time¹¹². The risk of recurrence with LAA is particularly high immediately following the initial clinical presentation but declines thereafter¹¹³. CE related stroke was associated

with the highest risk of recurrence of any aetiological types in longer term follow up ¹¹¹. Thus, whilst very early recurrence may be less in CE compared with LAA-related stroke (the limitations of incomplete investigation for AF and consideration of CE related stroke as a single entity accepted), medium and long term CE related risk may exceed that for LAA.

The discussion below details the risk of recurrence associated with the various aetiological subtypes of ischaemic stroke. It is noteworthy that less data are available for the TIA population ¹¹⁴. As described above, the risk of early recurrence following TIA is higher than following ischaemic stroke. Therefore identification and optimised treatment of aetiological factors in TIA patients may be more pressing than for ischaemic stroke patients.

1.3.6.1. Presumed arterial aetiology

The PROFESS study population comprised patients with ischaemic stroke or TIA (mean age 66 years), of presumed arterial origin (only 2.7% of those enrolled had AF). Patients were treated with current optimal antiplatelet therapy and high levels of concomitant antihypertensive and statin therapy. During a mean follow up period of 2.5 years, 8.8% of control (Clopidogrel) patients experienced a recurrent stroke, whilst 13.1% suffered a secondary composite outcome of stroke, MI or vascular death ¹¹⁵.

The SPARCL study population comprised patients with previous ischaemic stroke or TIA (mean age 63 years) of presumed arterial aetiology, with exclusion of patients with cardioembolic mechanisms for stroke. During a mean follow up period of 4.9 years, 11.2% and 14.1% of patients in the active (Atorvastatin) group experienced a recurrent ischaemic stroke any major cardiovascular event, respectively ¹¹⁶.

1.3.6.2. Small vessel disease

There is conflicting data regarding the risk of recurrent stroke following SVD related stroke. In a meta-analysis of retrospectively applied TOAST criteria to the OCSP, OXVASC, Erlangen & Rochester stroke registries, 7-day recurrence was observed in no patients, whilst 30-day and

1-year recurrence were observed in only 2.0% (95% CI, 0-4.2%) and 3.4% (95% CI, 0.6-6.3%) of patients, respectively ¹¹⁰. In contrast to the findings reported by Lovett, the rate of recurrence in patients with SVD in the PRoFESS study was 8.3% after 2.5 years of follow up, which was comparable to the combined rate in patients with LAA, undetermined, other & cardioembolism related stroke ¹¹⁵.

1.3.6.3. Large artery atherosclerosis

Patients with LAA are at high risk of stroke recurrence, with the greatest risk observed in the period immediately following ischaemic or TIA. In the Rochester population, 18.5% of patients experienced 30-day recurrence, rising to 24.2% at 1-year and 29.3% 2-years ¹¹⁷. Similar event rates were reported in the NOMASS population, with 14.4% suffering recurrence within 30-days, rising to 26.5% at 1-year ¹¹⁸. Clinical trials which evaluated the role of surgical intervention in patients with 50-69% and $\geq 70\%$ carotid atherosclerotic stenotic disease, reported a combined endpoint at 5 year follow-up of an ipsilateral stroke and any 30-day stroke or death, in 15.9% and 24.4% of medically managed control group patients, respectively ¹¹³. Risk increases with the degree of arterial stenosis ¹¹⁹.

There is evidence that much of this risk is attributable to recurrent events in the initial weeks and months following the index event and decays thereafter ^{120, 121}. The risk of a recurrent stroke amongst patients awaiting carotid endarterectomy (CEA) surgery is estimated at 5% per week in the initial weeks following stroke ¹²². In the ECST, which reported annual events rates for a mean follow-up duration of 6.1 years, an exponential decline in events was observed with time, with the majority of events occurring within the initial 3 years of follow up and the highest risk period within the initial 12 months ^{121, 123}. The lower rates in later years were not accounted for by early mortality amongst those patients with more severe stenosis.

1.3.6.4. Cardioembolic stroke and atrial fibrillation

AF represents the main cause of cardioembolic stroke. However, assignment of risk of recurrence to AF as an aetiological factor is challenging for several reasons:

Firstly, AF is frequently reported as a component of one of two TOAST categories, either cardioembolic or undetermined pathology. In the former category, AF represents a single component of all cardioembolic pathologies included in a composite definition, despite the likelihood that risk varies according to the specific underlying cardiac abnormality. Thus the risk attributable to the commonest cardioembolic cause of stroke, AF, may be inappropriately estimated when reported as a composite average with non-AF cardioembolic aetiological factors (e.g. an isolated finding of a PFO). The same difficulty arises when AF is considered as a component of the latter category, undetermined culprit pathology. This group is likely to include patients with heterogeneous risk, ranging from very high risk in the context of dual or multiple pathologies (e.g. coexisting AF and LAA), to low risk in the context of “true” cryptogenic stroke, defined following extensive investigation.

Secondly, there has been geographical and temporal variation in the approach to investigation to identify potential aetiological factors for stroke. The diagnosis of presence of AF in many of the early population studies was based on a sole 12-lead ECG, performed at the point of clinical presentation¹²⁴. The prevalence of (and risk attributable to) paroxysmal AF (PAF) may potentially have been incorrectly estimated in studies which did not employ more prolonged cardiac monitoring.

Thirdly, population characteristics influence the observed risk of recurrence. In particular, the age of the population affects the risk of embolic events in patients with AF, independently of the increased prevalence of AF with aging. Accordingly, clinical trials, which historically limited the upper age of eligibility, are limited in assessing risk of recurrence. Similarly, population studies reporting risk as a composite of all age groups are likely to inappropriately describe risk for age subgroups. AF may account for 20% of all strokes but likely accounts for a greater proportion in the more elderly. The elderly population is increasing and therefore AF is likely to hold a proportionally greater responsibility for the burden of stroke overall in the decades to come.

Patients with AF have traditionally been considered to be at particularly elevated risk of early stroke recurrence. In small study populations, the risk of early recurrence in patients with ischaemic stroke and AF has been reported to be as high as 33% within 2.3 months ¹²⁵. In another study, 20% of patients with AF suffered recurrent embolism within 11 days of their index event ¹²⁶. In a meta-analysis of the OCSP, OXVASC, Erlangen & Rochester registries, 7-day recurrence was observed in 2.5% (95% CI, 0.1 – 4.9%) of patients with cardioembolic stroke, whilst 30-day and 1-year recurrence were observed in 4.6% (95% CI, 1.3-7.9) and 11.9% (95% CI, 6.4-17.4%) of patients, respectively ¹¹⁰. More detailed breakdown of risk within the cardioembolic group was not reported. Whilst some observational studies reported similar risk of recurrence in AF as for patients in sinus rhythm (this likely reflected the comparator being all non-AF stroke combined, including those with other high risk aetiological factors e.g. LAA) ¹²⁴, the majority of population based studies have identified an increased early and long term risk of stroke recurrence in patients with AF ¹²⁷⁻¹²⁹. In population studies, the early risk (within 2 weeks) of recurrence of stroke in patients with AF has been variably estimated at between 0.3 – 1.1% per day ^{127, 129-131}.

In ischaemic stroke clinical trial populations, the risk of early recurrence has been variably reported. In the IST (mean age 77.8 years), ischaemic stroke recurrence occurred in 4.9% in patients with AF not treated with UFH within 14 days ⁵⁶. In the HAEST study (mean age 80 years), 14-day ischaemic stroke recurrence was higher, observed in 8% of patients ⁵⁸. A meta-analysis of acute stroke patients with AF enrolled in RCTs reported a stroke recurrence rate of 5% within the first 2 weeks following the qualifying event ¹³². Contrasting early recurrence rates in unselected stroke patient trial populations with no diagnosis of AF (3.8% of patients at 14-days in the IST, 1.3% of patients at 7-days in TOAST and 2.6% of patients at 30-days in CAST ^{53, 54, 57}), it is apparent that an excess of risk in patients with AF might present an opportunity for expedited diagnosis of AF and initiation of optimised anticoagulant based secondary prevention.

Beyond the risk of early recurrence, patients with AF remain at very high intermediate and long term risk of ischaemic stroke recurrence. Marini and colleagues reported higher 1 year recurrence amongst patients with admission AF compared to those without AF ¹³³. Whilst AF independently increases the risk of stroke five-fold in the general population ¹³⁴, in patients

with prior ischaemic stroke or TIA, the risk of stroke / SE / major bleeding / ICH & mortality is increased a further two to threefold ^{135, 136}. In population studies, recurrence rates between 2 – 15% are quoted for the first year following presenting stroke and 5% each year thereafter ⁹¹. Higher rates of recurrence were observed in some populations ¹²⁷. The EAFT enrolled patients with AF (mean age 73 years), though patients with other concomitant, potentially culprit pathologies were not excluded. During a mean follow up period of 2.3 years, 12% of placebo control patients experienced a recurrent stroke ¹³⁷. 4% of coumarin treated patients experienced an event. The majority of clinical events were observed in the initial 12 months of EAFT follow up. The elevation in risk of recurrence associated with AF appears to be irrespective of the aetiological mechanism for the incident stroke ¹³⁸.

1.4. Secondary prevention of ischaemic stroke following ischaemic stroke & TIA

Given the high levels of incomplete recovery and the high risk of stroke and other cardiovascular events following stroke and TIA, implementation of optimal preventative strategies represents a cornerstone of the management of cerebrovascular disease.

1.4.1. Lifestyle modification

Guidelines recommend smoking cessation, limited alcohol consumption, weight loss in obesity and regular physical exercise as lifestyle modifications likely to confer beneficial effects in reduced risk of stroke recurrence ¹⁹.

1.4.2. Antihypertensive therapy

An aetiological relationship between BP and risk of stroke has been established in population studies and clinical trials ¹³⁹. The relationship appears linear, with changes in BP even at low and “normal” levels associated with modified risk. In the context of primary prevention, antihypertensive treatment dramatically reduces the risk of stroke, irrespective of the type of agent used ¹⁴⁰.

Less patient data is available in the context of secondary prevention, particularly regarding reduction of BP in the immediate aftermath of ischaemic stroke. However, meta-analysis of available trial data confirms a significant benefit of BP reduction with antihypertensive therapy, commenced between 3 weeks to 14 months following stroke or TIA ¹⁴¹. Recurrent stroke (RR 0.75, 95% CI 0.63 – 0.92) and all vascular events (RR 0.79, 95% CI 0.66 – 0.95) were each reduced with antihypertensive therapy. This analysis identified independent benefit of both Thiazide monotherapy and combination therapy with angiotensin converting enzyme inhibitor (ACE-I) therapy. Other antihypertensive strategies were not significantly associated with benefit, though confidence intervals were wide. The greater the reduction in BP

observed, the greater the reduction in clinical events. Direct interclass antihypertensive trials in the setting of secondary prevention following stroke and TIA are lacking.

The observed benefit for Thiazide and Thiazide / ACE-I combination therapy was driven by the results of the PROGRESS study ¹⁴². In this placebo controlled trial, 6105 patients with stroke or TIA within the preceding 5 years, were randomised to receive the ACE-I Perindopril 4mg daily plus the Thiazide diuretic Indapamide 2.5mg daily, compared with placebo. Blood pressure was significantly reduced by 9/4 mmHg with active treatment over 4 year follow-up. Both stroke RRR 28% (95% CI, 17 – 38%) and all major vascular events RRR 26% (95% CI, 16 – 34%), were each significantly reduced with active treatment. On subgroup analysis, statistically significant benefit was only observed amongst patients in the active group treated with combination therapy, in whom the BP reduction observed was 12/5 mmHg, compared with 5/3 mmHg for Perindopril monotherapy.

In a factorial design, The PROfESS study compared the angiotensin receptor blocker (ARB) Telmisartan with Placebo in 20,332 patients with ischaemic stroke ¹⁴³. Despite a significantly greater reduction in systolic BP (of 4 mmHg) with Telmisartan treatment, no difference in either stroke recurrence or combined vascular events was observed.

Although the PROfESS trial was underpowered and commentators have noted that a treatment difference may have emerged with more prolonged follow up, the study failed to support the specific use of ARB treatment following stroke. Taken together with the PROGRESS study, which also failed to demonstrate clear benefit with a modest BP reduction with ACE-I monotherapy, the results of secondary preventative data meta-analysis ¹⁴¹ and other BP reduction trials ¹⁴⁴, a clear conclusion appears to be that the magnitude of clinical benefit appears to be related to the magnitude of BP reduction, with a mean reduction of 10/5 mmHg being clinically effective ¹⁹. The available data support combination therapy with ACE-I and thiazide diuretic as an initial choice in treatment.

1.4.3. Lipid lowering therapy

The epidemiological association between raised LDL-cholesterol and increased risk of stroke is less apparent than for CHD ¹⁴⁵. In addition, haemorrhagic stroke risk, evident as microbleeds detected on gradient echo sequence MRI, may be increased at very low levels of total-cholesterol and the highest levels of HDL-cholesterol ¹⁴⁶. Though evidence for benefit of statin therapy in reducing risk of stroke exists in CHD patient populations ¹⁴⁷, randomised evidence for benefit of lipid lowering with statin therapy following ischaemic stroke has only emerged in the last decade.

In the Heart Protection Study, treatment with Simvastatin 40mg daily significantly reduced a composite endpoint of all major vascular events amongst a subgroup of 3280 patients with a remote history (mean 4.2 years) of ischaemic cerebrovascular disease ¹⁴⁸.

In the SPARCL study, 4731 patients with recent (1 – 6 months) stroke or TIA, were treated with Atorvastatin 80mg daily or placebo ¹¹⁶. The study excluded patients with stroke of presumed cardioembolic aetiology and patients had no prior diagnosis of CHD. Over a median of 4.9 year follow-up, both recurrent stroke (of any type) and combined vascular events, were significantly reduced with Atorvastatin, HR 0.84 (95% CI, 0.71 – 0.99) and HR 0.80 (95%CI, 0.69 – 0.92), respectively. The absolute risk reductions for these endpoints were 2.2% and 3.5%, respectively. A significant increase in risk of haemorrhagic stroke was observed with Atorvastatin (HR 1.66, 95% CI 1.08 – 2.55), which was outweighed by the reduction in ischaemic events.

Though the increased risk of subsequent haemorrhagic stroke with statin therapy is outweighed by the reduction in ischaemic stroke and CHD events, it has implications in considering the utility of secondary preventative statin therapy in patients without dyslipidaemia or other clinical indications for statin therapy following ICH stroke ¹⁹.

1.4.4. Hypoglycaemic therapy

Diabetes mellitus is an independent risk factor for stroke ^{149, 150}. There is evidence in primary prevention that reductions in BP and LDL-cholesterol are beneficial in reducing the incidence

of stroke. The benefits of aggressive glycaemic control in primary prevention have been less apparent ¹⁵¹. In secondary prevention studies examining glycaemic control, aggressive and intensive lowering of blood sugar with a view to achieving lower HbA1C concentrations has failed to demonstrate benefit in reduction in macrovascular events, including stroke and in some cases evidence of harm was observed ¹⁵².

The PROactive trial compared Pioglitazone with placebo in patients with a history of macrovascular disease and included a subgroup of 984 patients with prior stroke ¹⁵³. Active treatment was associated with a significant reduction in the secondary endpoints of any stroke [HR 0.53 (95% CI 0.34 – 0.85)] and composite of vascular death, non-fatal MI & non-fatal stroke [HR 0.72 (95%CI 0.52 – 1.00)]. A trend toward a reduction in the primary endpoint of death and major vascular events was reported [HR 0.78 (95% CI 0.60 – 1.02)]. The IRIS clinical trial, comparing Pioglitazone with placebo in a population with previous stroke and TIA, is examining whether these secondary endpoint findings are reproducible.

1.4.5. Carotid intervention

Three clinical trials compared conservative, medical management with surgical carotid endarterectomy (CEA), in the management of internal carotid stenosis in patients with TIA and non-disabling ischaemic stroke: the ECST ¹²³, NASCET ¹²⁰ & and VACS ¹⁵⁴ studies. Each study suggested benefit of CEA over medical therapy. However, methodological differences in assessment of the degree of carotid stenosis created doubt regarding patient criteria defining suitability for surgery rather than medical management.

In 2003, an individual patient data meta-analysis was published based on re-categorisation of the degree of stenosis, based on the NASCET method, for patients in all three trials ¹¹⁹. This analysis reported that surgical benefit was limited to patients with $\geq 50\%$ stenosis. An absolute risk reduction (ARR) for 5-year ipsilateral ischaemic stroke of 4.6%, $p=0.04$, was observed for 50 – 69% stenosis; An ARR of 16.0%, $p<0.001$, was observed for $\geq 70\%$ stenosis without near occlusion. A subsequent sub-group analysis refined the patient criteria for which benefit of CEA exceeded medical therapy ¹¹³: Amongst female patients, benefit was limited to $\geq 70\%$ stenosis (without near occlusion); benefit of CEA declined with time:

patients with $\geq 70\%$ stenosis (without near occlusion) only benefited if surgery was performed within 12 weeks and patients with 50-69% stenosis only benefited if surgery was performed within 2 weeks of the index symptoms.

Not all patients are eligible for treatment with CEA, due to perceived excessive operative risk. The potential for percutaneous carotid artery stenting to deliver minimally invasive benefit led to the International Carotid Stenting Study which compared CEA with stenting. Long term follow up results are awaited but 120-day follow up data suggest significantly poorer outcomes (combined MI, stroke, death) amongst patients managed with stenting [HR 1.69 (95% CI, 1.16 – 2.45)]¹⁵⁵.

1.4.6. Antiplatelet therapy in presumed arterial & cryptogenic stroke

Antiplatelet therapy carries a substantial evidence base for beneficial reduction in cardiovascular events following ischaemic stroke or TIA¹⁵⁶⁻¹⁵⁹. Three antiplatelet agents recommended in secondary prevention are ASA, Dipyridamole and Clopidogrel^{19, 79}. Ticlopidine has also been established as effective in the secondary prevention of ischaemic stroke but is not recommended for use due to its adverse event profile¹⁹. A meta-analysis of trial data examining antiplatelet agents in patients with prior history of stroke demonstrated a 22% reduction in combined endpoint of stroke, myocardial infarction (MI) and vascular death (mean follow up duration 29 months), principally due to a significant reduction in non-fatal recurrent stroke¹⁵⁷.

Evidence for benefit for both ASA and Dipyridamole as monotherapies and of additive benefit for combination therapy, has been derived from ESPS, ESPS-2 and ESPRIT studies. In the ESPS-1 study, the combination of ASA with Dipyridamole was associated with a RR reduction in stroke or death of 33%, $p < 0.001$ (mean duration 24 months), compared with placebo¹⁶⁰. The ESPS-2 study, in patients with prior ischaemic stroke or TIA, was a 2 by 2 factorial design which compared the combination of ASA & Dipyridamole, ASA alone, Dipyridamole alone and placebo¹⁶¹. Compared with placebo, the RRR for stroke was 18.1% with ASA ($p = 0.013$), 16% with Dipyridamole ($p = 0.039$) and 37% with combination therapy ($p < 0.001$). Compared with ASA alone, the RRR for stroke was 23.1% with combination

therapy ($p=0.006$). In the unblinded ESPRIT study, compared with ASA monotherapy, ASA & Dipyridamole combination therapy significantly reduced the combined risk of stroke, MI, vascular death & major bleeding events, at 3.5 years (HR 0.80; 95% CI 0.66 to 0.98) ¹⁶².

In the CAPRIE study, Clopidogrel was compared with ASA for the prevention of a combined endpoint of MI, stroke & death ¹⁶³. The study population included patients at increased risk of CV events, including a subgroup with prior stroke. In the study population as a whole, Clopidogrel reduced annual CV events compared with ASA with borderline statistical significance (RRR 8.7%; 95% CI, 0.3 – 16.5%, $p=0.043$). This finding was driven by a reduction in events amongst patients with peripheral arterial disease. Amongst patients with prior stroke, no significant difference was observed between treatment groups (RRR 7.3%; 95% CI, -6 – 19%, $p=0.26$), though this subgroup analysis lacked statistical power to identify a treatment effect.

The observation that Clopidogrel may hold some additional benefit to ASA monotherapy whilst potentially inferior to the combination of ASA and Dipyridamole, led to the conduct of the PROFESS study ¹¹⁵. In a non-inferiority design, patients with prior ischaemic stroke (or TIA with acute brain imaging lesion) were randomised to receive either Clopidogrel monotherapy or the combination of ASA and MR-Dipyridamole. No significant difference was observed in recurrent ischaemic stroke between treatment groups (mean duration 2.5 years) (HR 1.01; 95% CI, 0.92 to 1.11), or for any stroke (HR 1.05; 95% CI, 0.96 – 1.16). A non-significant trend toward increased bleeding events was observed with combination therapy (HR 1.15; 95% CI, 1.00 – 1.32), which was also associated with reduced concordance with treatment due to side effects. The hypothesis of non-inferiority of ASA & Dipyridamole combination to Clopidogrel alone was not rejected due to the size of the observed confidence intervals (which crossed the upper limit of the non-inferiority margin). However survival curves indicated that the two strategies were equivalent during the observed period of follow-up. Testing for treatment interaction between LAA and SVD aetiological subgroups was non-significant ($P > 0.05$). Clinical guidelines have subsequently recommended either ASA in combination with Dipyridamole-MR, or Clopidogrel monotherapy as valid long term antiplatelet secondary preventative strategies ^{19, 79}.

The combination of ASA with Clopidogrel has been studied in several trials, to evaluate whether similar benefits as have been observed in the CHD population with combination therapy, are evident following ischaemic stroke or TIA. The MATCH study, in patients with prior TIA or ischaemic stroke, compared the combination of ASA & Clopidogrel with Clopidogrel alone¹⁶⁴. During a mean follow-up of 3.5 years, combination therapy significantly increased bleeding events without a reduction in cardiovascular events compared with Clopidogrel alone. In the CHARISMA study, the combination of ASA & Clopidogrel was evaluated, in comparison with ASA alone. The study population included patients with CVD or multiple risk factors. No significant treatment difference (in MI, stroke, CV death) was observed in the overall trial population or in a subgroup of patients with previous stroke. An excess of bleeding events with combination therapy was again observed¹⁶⁵. More recently, in a Chinese population of patients with acute mild severity ischaemic stroke or TIA, the combination of ASA & Clopidogrel for 21 days followed by Clopidogrel to 90 days reduced recurrent stroke compared with ASA monotherapy (HR 0.68; 95% CI, 0.57 to 0.81; P<0.001), with similar bleeding complications observed between treatment groups⁵⁵.

1.4.7. Anticoagulant therapy in presumed arterial & cryptogenic stroke

Anticoagulant therapy has been evaluated in patients with presumed arterial and cryptogenic stroke. The ESPRIT trial compared warfarin (with a target INR of 2 – 3) with antiplatelet therapy (either ASA monotherapy or combination of ASA / MR-Dipyridamole). A borderline significant reduction in ischaemic events was observed with warfarin compared with ASA monotherapy (HR 0.73; 95% CI 0.52 – 1.01) but also with increased bleeding (HR 2.56; 95% CI 1.48 – 4.43)¹⁶⁶. The SPIRIT study evaluated higher target INR anticoagulation compared with ASA and was terminated early due to excess bleeding risk with anticoagulation¹⁶⁷. Warfarin, with a target INR of 1.4 – 2.8, was compared with ASA 325mg od, in the WARSS study¹⁶⁸. In patients with non-cardioembolic stroke, no significant difference was observed between treatment groups for either stroke or death (warfarin 17.8%, ASA 16.0%) or bleeding events (warfarin 2.2%, ASA 1.5%). The WASID study compared warfarin (target INR 2.0 – 3.0) with ASA in patients with intracranial atherosclerosis¹⁶⁹. No difference in ischaemic stroke / ICH /

death was observed with treatment but extracranial bleeding was increased in the warfarin group.

A meta-analysis has been conducted ¹⁷⁰ examining anticoagulant use in minor ischaemic stroke and TIA of presumed arterial origin compared with antiplatelet therapy. Recurrent ischaemic stroke was similar with medium intensity anticoagulation [RR 0.80, 95% confidence interval (CI) 0.56 to 1.14] or high intensity anticoagulation [RR 1.02, 95% CI 0.49 to 2.13], compared with antiplatelet therapy. Medium intensity (RR 1.93, 95% CI 1.27 to 2.94) and high intensity (RR 9.0, 95% CI 3.9 to 21) anticoagulation were each associated with a higher risk of major bleeding complications compared with antiplatelet therapy. Guidelines recommend antiplatelet agents for secondary prevention rather than anticoagulants in patients with non-cardioembolic stroke ¹⁹.

1.4.8. Antiplatelet therapy in ischaemic stroke & TIA patients with AF

Antiplatelet therapy in patients with non-valvular AF has been found to be effective in reducing recurrent stroke in studies including both primary and secondary prevention populations. Absolute benefit is greater in the latter such that there is debate regarding utility of antiplatelet agents in the primary prevention population with low risk of stroke, due to the hazard for bleeding complications.

In a Cochrane library systematic review and meta-analysis of trials evaluating ASA in patients with AF but no prior history of stroke or TIA, amongst 1965 patients followed up for a mean of 1.3 years, a non-significant reduction in all strokes (OR 0.70; 95% CI, 0.46 – 1.07) and ischaemic stroke (OR 0.70; 95% CI, 0.46 – 1.07), with ASA therapy was observed ¹⁷¹. A composite outcome of all stroke, MI or vascular death was significantly reduced with ASA therapy (OR 0.71; 95% CI 0.51 – 0.97). The authors concluded that ASA likely reduces stroke and vascular events in patients with non-valvular AF but only modestly so. The annualised rate of ischaemic stroke in the placebo treated patients of approximately 4% per year suggests that 12 ischaemic strokes would be prevented yearly for every 1000 patients treated with ASA.

The EAFT included patients with AF and a recent TIA or mild ischaemic stroke of any aetiological type, excluding only patients scheduled for imminent carotid & coronary surgery¹³⁷. The study included two arms. The first randomised patients to receive adjusted dose warfarin, ASA or placebo. A second arm randomised patients considered unsuitable for anticoagulation in some regard, to receive either ASA or Placebo. 40% of patients were randomised within 14-days of their qualifying event. Outcomes in ASA treated patients were similar in the two arms and the authors elected to report the ASA – Placebo comparison in the two arms as composite data. Compared with placebo, ASA non-significantly reduced any stroke (HR-adjusted 0.86; 95% CI, 0.64 – 1.15) and combined vascular events (HR 0.83; 95% CI, 0.65 – 1.05). The lack of statistical benefit observed with ASA in relation to recurrent stroke, may have reflected a lack of efficacy in preventing AF-related cardioembolism and lack of statistical power to detect the treatment effect that would typically be anticipated in relation to non-cardioembolic stroke mechanisms.

A meta-analysis from the ATTC considered the effect of ASA in combined primary and secondary preventative groups and reported a significant 25% reduction in the risk of stroke¹⁷². A subsequent meta-analysis, again including both primary and secondary prevention patients reported a 22% RRR for any stroke (95% CI, 2 – 39%) with ASA compared with placebo and 22% RRR for any stroke (95% CI 6 – 35%) for any antiplatelet¹⁷³.

In the ACTIVE-A study, patients with non-valvular AF, considered unsuitable for formal anticoagulant therapy, were randomised to receive Clopidogrel or Placebo in addition to ASA¹⁷⁴. Over median 3.6 years follow-up, this study demonstrated superiority of combined antiplatelet therapy to ASA alone in reducing stroke event (2.4 versus 3.3% per year, RR 0.72; 95% CI, 0.62 – 0.83) but at the expense of increased major bleeding complications (2.0% versus 1.3% per year, RR 1.57; 95% CI 1.29 – 1.92) .

1.4.9. Anticoagulant therapy in ischaemic stroke & TIA patients with AF

1.4.9.1. Anticoagulant therapy compared with placebo

A Cochrane library systematic review and meta-analysis of trials evaluating adjusted dose anticoagulation with vitamin K antagonists (VKA) in patients with AF but no prior history of stroke or TIA was published in 2005. Data for 2313 patients enrolled in five trials were included, with mean follow-up of 1.5 years. The mean patient age in this analysis was 69 years, with only 20% of participants aged over 75 years. The authors reported a reduction in all strokes (OR 0.39; 95% CI, 0.26 – 0.59), ischaemic stroke (OR 0.34; 95% CI, 0.23 – 0.52) and all-cause mortality (OR 0.69; 95% CI 0.50 – 0.94) with anticoagulant therapy ¹⁷⁵.

In the EAFT secondary prevention study, compared with placebo, warfarin significantly reduced any stroke (HR-adjusted 0.34; 95% CI, 0.20 – 0.57) and combined vascular events (HR 0.53; 95% CI, 0.36 – 0.79) ¹³⁷. Data from the EAFT were combined with those from patients with prior stroke included in the VA-SPINAF study in a Cochrane systematic review assessing adjusted dose anticoagulation compared with placebo in patients with NRAF and prior history of stroke or TIA ¹⁷⁶. Recurrent stroke (any type) (OR 0.36; 95% CI, 0.22 – 0.58) and all vascular events (stroke, MI, SE, vascular death) (OR 0.55; 95% CI 0.37 – 0.82) were each significantly reduced with anticoagulant therapy. Major extracranial haemorrhage was increased with anticoagulant therapy (OR 4.32; 95% CI 1.55 – 12.10).

The RR reductions with anticoagulant therapy compared with placebo were similar in both primary and secondary prevention populations. However, absolute risk reductions differ. In patients without prior stroke, the annualised ischaemic stroke rate in the control group was approximately 4% per year. The absolute reduction in stroke with anticoagulation was approximately 2.6% per year (or 25 ischaemic strokes prevented yearly per 1000 treated patients) ¹⁷⁵. In contrast, the annual incidence in patients with prior stroke or TIA was 12% per year ¹⁷⁶. 90 vascular events (mainly strokes) are prevented if 1000 patients are treated for 1 year ¹⁷⁶. A subsequent meta-analysis reported an ARR, in any stroke, of 8.4% per year in patients with prior stroke or TIA and 2.7% per year in the context of primary prevention, for adjusted dose anticoagulation compared with placebo ¹⁷³.

It is noteworthy that the risk of ischaemic stroke is significantly increased amongst patients who experience subtherapeutic INR control. Compared with patients with an INR of 2.0 or higher, the OR for ischaemic stroke in patients with an INR or 1.5 or less is 3.3 (95% CI, 2.4 – 4.6)¹⁷⁷. Thus, the benefits of anticoagulation are reliant on adequate control within the therapeutic range.

1.4.9.2. Anticoagulant therapy compared with antiplatelet therapy

An individual patient data meta-analysis of clinical trial data, in 986 patients with prior stroke or TIA, reported annual ischaemic stroke event rate of 10.0% per 100 patient years with ASA therapy compared with 4.0% on adjusted dose oral anticoagulation¹⁷⁸. In 3,066 patients without prior history of stroke or TIA, the annual ischaemic stroke event rate was 2.7% per 100 patient years with ASA therapy compared with 1.5% on adjusted dose oral anticoagulation. Two Cochrane reviews have subsequently compared adjusted dose anticoagulation with antiplatelet therapy in patients without and with, prior history of ischaemic stroke or TIA.

A Cochrane library systematic review and meta-analysis of trials evaluating adjusted dose anticoagulation with VKA, compared with antiplatelet therapy, in patients with AF but predominantly (~90%) no prior history of stroke or TIA, was published in 2007¹⁷⁹. Data for 9598 patients enrolled in five trials were included, with mean follow-up of 1.9 years. The authors reported a significant reduction in all strokes (OR 0.68; 95% CI, 0.54 – 0.85), ischaemic stroke (OR 0.53; 95% CI, 0.41 – 0.68), systemic embolic events (OR 0.48; 95% CI, 0.25 – 0.90), a composite outcome of all stroke, MI or vascular death (OR 0.74; 95% CI, 0.61 – 0.90) and all-cause mortality (OR 0.74; 95% CI 0.61 – 0.91), with anticoagulant compared with ASA therapy.

The seminal EAFT is the only randomised clinical trial to have specifically compared adjusted dose anticoagulation with ASA in the context of ischaemic stroke and TIA secondary prevention¹³⁷. Compared with ASA, adjusted dose warfarin significantly reduced all stroke (HR-adjusted 0.38; 95% CI, 0.23 – 0.64) and combined vascular events (HR 0.60; 95% CI, 0.41

– 0.87). Survival curves continued to diverge at the point of trial termination of follow-up, suggesting that treatment ought to continue indefinitely.

A Cochrane library systematic review and meta-analysis of trials evaluating adjusted dose anticoagulation with VKA compared with antiplatelet therapy in patients with AF and with prior history of stroke or TIA was published in 2004 and included data for 1371 patients enrolled in two trials; the EAFT and the SIFA (in which the antiplatelet agent studied was Indobufen)¹⁸⁰. The authors reported a significant reduction in all stroke (Peto OR 0.49; 95% CI, 0.33 – 0.72) and all vascular events (Peto OR 0.67; 95% CI, 0.50 – 0.91), compared with antiplatelet therapy. Major extracranial bleeding was increased with anticoagulant compared with antiplatelet therapy (Peto OR 5.16; 95% CI, 2.08 – 12.83).

Based on an annualised rate of ischaemic stroke of 4% per year on antiplatelet therapy in the primary prevention population, approximately 19 events per year would be prevented for every 1000 patients treated with anticoagulation rather than antiplatelet¹⁷⁹. In contrast, in the EAFT, the annual rate for any stroke in ASA treated patients was 10% per year. The rate in anticoagulated patients was approximately 4%, so that anticoagulation is associated with 60 fewer recurrent strokes per year per 1000 patients treated, compared with ASA¹⁸⁰.

A subsequent meta-analysis, which included the results of the large ACTIVE-W study, reported an RRR for any stroke of 37% (95% CI, 23 – 48) for all trials comparing adjusted dose anticoagulation with antiplatelet therapy. The ARR for any stroke, with adjusted dose anticoagulation compared with ASA, was 7.0% per year in patients with prior stroke or TIA and 0.7% per year in the context of primary prevention¹⁷³.

In the ACTIVE-W study, adjusted dose Warfarin was compared with combination of ASA & Clopidogrel¹⁸¹. The study was discontinued prematurely due to the superiority of adjusted dose warfarin (RR 1.44; 95% CI 1.18 – 1.76). Studies assessing fixed-low-dose anticoagulation, in combination with ASA, have failed to demonstrate a significant reduction in embolic events, whilst bleeding was increased¹⁸². Studies targeting higher range INR were associated with excess bleeding¹⁸³.

The AVERROES trial randomised patients with AF at increased risk of stroke but unsuitable for treatment with VKA, to receive either Apixaban or ASA¹⁸⁴. During mean follow-up of 1.1

years, stroke & SE were observed less frequently with Apixaban than ASA (HR 0.45; 95% CI 0.32 – 0.62). Major bleeding events were similar in the two treatment groups (HR 1.13; 95% CI, 0.74 – 1.75). Treatment effects were consistent in important subgroups, including patients with prior stroke or TIA (although there was a trend toward significantly greater benefit of Apixaban in this group).

Following on from the evidence for superiority of anticoagulation over antiplatelet therapy in AF for both primary and secondary stroke prevention in younger adults, the BAFTA study enrolled 973 patients aged 75 years and over¹⁸⁵. During mean 2.7 years follow up, a composite endpoint of fatal / disabling stroke, ICH and arterial embolism was significantly lower in warfarin treated patients compared with ASA RR 0.48; 95% CI 0.28 – 0.80).

1.4.9.3. The optimal choice of anticoagulant agent

The applicability of benefits of anticoagulant therapy demonstrated within clinical trials may not be universal. Many authors and clinicians believe the balance of risk and benefit may be completely reversed in particular patient groups. Real life populations are often more elderly, frail with multiple co-morbidities including cognitive impairment. These combine to make the practicalities of therapeutic monitoring more challenging and increase the risk of either under or over anticoagulation (with adherent risks of bleeding complications).

The utility of anticoagulation in patients with AF could be optimised^{186, 187}. Treatment appears to be underutilised in the wider population, potentially due to either patient or physician (or a combination of both) reluctance to use warfarin¹⁸⁸. In one study, approximately half of patients with no apparent contraindication were found to be untreated¹⁸⁶. Perceived risks of haemorrhages and practicalities are likely contributing factors to such reluctance¹⁸⁹. The current proportion of patients with identifiable AF following stroke typically commenced on anticoagulant treatment is only 39%²³. Whilst it is unlikely that all stroke patients with AF will ever be suitable for anticoagulant therapy, availability of new direct thrombin and factor Xa inhibitors will greatly increase the proportion that will safely benefit from reduced embolic phenomena through treatment.

Many patients on treatment in the community are found to be inadequately anticoagulated ¹⁸⁸. This also occurs in closely monitored clinical trial populations ¹³⁷. Treatment outwith the therapeutic INR is associated with increased risk of clinical events ^{177, 190}. Prolonged periods of over anticoagulation are associated with increased risk of bleeding events ¹⁹¹.

Lack of universal applicability of warfarin therapy, together with practical challenges of monitoring and maintaining patients within the target range of anticoagulation, has prompted exploration of alternative novel anticoagulant agents (NOACs), which confer predictable anticoagulant effects.

A subgroup of 3,436 patients with prior ischaemic or TIA were included in the ARISTOTLE trial ^{135, 192}. The stroke population included was neither restricted nor enriched according to TOAST diagnostic criteria. Over median follow-up of 1.8 years, 4.0% of patients treated with Apixaban suffered any stroke compared with 5.5% of patients treated with warfarin (HR 0.71; 95% CI, 0.52 – 0.98). ICH was lower in Apixaban treated patients (HR 0.40; 95% CI, 0.21 – 0.78). Observed results in this subgroup were consistent with those in non-stroke patients across all endpoints. In the ROCKET-AF study, Rivaroxaban was found to be non-inferior to adjusted dose warfarin in patients with prior ischaemic stroke or TIA ¹⁹³ and in the RE-LY study, Dabigatran was found to be non-inferior to adjusted dose VKA for stroke reduction, with an associated small excess in MI ¹⁹⁴.

A recent meta-analysis comparing NOAC agents with vitamin K antagonist therapy, identified a significant reduction in all stroke (RR 0.81, 95% CI 0.73—0.91) with the NOAC agents. Intracranial bleeding was reduced with NOAC agents though there was a significant increase in the risk of gastrointestinal bleeding ¹⁹⁵. Guidelines are being updated to reflect the emerging evidence base regarding NOAC agents in relation to VKA and antiplatelet therapy. It appears likely that these agents will emerge as treatments of choice in relation to cardioembolic prophylaxis.

1.4.10. Rate versus rhythm control strategies in AF

Several clinical trials have examined the merits of a “rhythm control” strategy, where the goal of treatment is to maintain the patient in sinus rhythm, as compared with a “rate control” strategy, in which AF is accepted as the prevailing cardiac rhythm with focus on reducing complications (tachy-cardiomyopathy, embolic phenomena).

The largest trial to compare these strategies was the AFFIRM study¹⁹⁶. 4060 patients (mean age 69.7 years) with AF aged over 65 years and at least one additional CV risk factor, were randomised to rhythm control (comprising DC cardioversion following a period of anticoagulation, followed by antiarrhythmic drugs) or rate control (with choice of medication at clinician discretion). Anticoagulant therapy was recommended unless otherwise contraindicated in the rate control group but could be discontinued at clinician discretion if successful rhythm control was perceived to have been achieved. During mean follow-up of 3.5 years, a non-significant trend toward higher mortality in the rhythm control group (23.8% versus 21.3%, $p=0.08$), was observed. During the study more than 85% those patients in the rate control group were maintained on coumadin, while in the rhythm control group that number declined to 70% after the first four months (& remained at that approximate level). Despite comprising a smaller number of patients, approximately twice as many strokes were observed in rhythm control patients as rate control patients who discontinued anticoagulation (44 versus 25 of 69 ischaemic strokes). A conclusion of the study was that whilst rhythm control may appear to have been achieved, risk of cardioembolic stroke persisted and discontinuation of anticoagulant therapy was associated with an excess of clinical events, which appeared to largely explain the observed trend in mortality in the rhythm control group.

Guidelines reflect these findings in recommending rhythm control for symptomatic morbidity¹⁹⁷. In patients in whom maintenance of sinus rhythm is unlikely to be successful, anticoagulation for cardioembolic prophylaxis should not be routinely discontinued if rhythm control is being pursued.

1.4.11. Non-pharmacological approaches to secondary prevention in AF

A proportion of patients may remain unsuitable (or unwilling) for anticoagulation with either warfarin or the novel oral anticoagulant agents (NOACs). Patients without prior stroke or other risk factors necessitating consideration of anticoagulation may experience symptomatic morbidity. These patient groups may be considered for non-anticoagulant based strategies by which to eliminate the occurrence of AF (rhythm control through either pharmacological or interventional procedures) or the embolic complications of AF (interventional procedures). In the PROTECT-AF study, placement of the WATCHMAN left atrial appendage occlusion device was evaluated versus adjusted dose warfarin. The authors reported that the WATCHMAN device may be non-inferior to adjusted dose INR warfarin therapy in reducing the risk of embolic events¹⁹⁸.

1.4.12. Cervical artery dissection

Following cervical artery dissection, the risk of stroke appears highest in the days immediately following the dissection¹⁹⁹. Beyond this, stroke rates appear low, estimated at approximately 0.3% between 3 – 12 months in two case series^{200, 201}.

There are limited data available to guide medical management following cervical artery dissection. A systematic review and meta-analysis comparing antiplatelet with anticoagulant therapy in carotid dissection reported a non-significant trend toward increased death or disability with antiplatelet compared with anticoagulant therapy [OR 1.94, 95% CI 0.79 – 4.91]²⁰². A separate meta-analysis included both carotid and vertebral arterial dissection cases and reported no difference in death or stroke rates between antiplatelet and anticoagulant therapy²⁰³. Neither of these systematic reviews included patients enrolled in randomised comparisons. Randomised trials are ongoing to address the optimal management strategy and no specific recommendations are made in current guidelines regarding the optimal choice of medical management¹⁹.

1.4.13. Cardioembolic stroke (other than related to AF)

1.4.13.1. Patent foramen ovale

A patent foramen ovale (PFO) may be present in up to 25% of the population ¹¹⁷. PFO may be associated with an atrial septal aneurysm and the latter may also exist in isolation. Epidemiological data have suggested PFO may be causally related to incident stroke, though data are conflicting.

A systematic review of population based data identified elevated risk of incident stroke in association with presence of PFO. In patients < 55 years of age, compared with patients with neither PFO nor atrial septal aneurysm, the OR for stroke in the presence of PFO, atrial septal aneurysm and PFO with atrial septal aneurysm were: 3.1 (95% CI, 2.29 – 4.21), 6.14 (95% CI, 2.47 – 15.22) and 15.59 (95% CI, 2.83 – 85.87), respectively ²⁰⁴. In patients > 55 years, a similar pattern of risk albeit of lower relative magnitude was observed.

A consecutive patient case-series study reported presence of PFO in a significantly greater proportion of patients with cryptogenic stroke than stroke of known cause, irrespective of patient age ²⁰⁵. In the European PFO-ASA study, in patients aged 18 – 55 years with stroke of unknown cause, 4-year stroke recurrence in patients with neither PFO nor atrial septal aneurysm was 4.2% (1.8 – 6.6). Stroke recurrence was lower in patients with PFO, 2.3% (0.3 – 4.3) and higher in patients with both PFO and atrial septal aneurysm, 15.2% (1.8 – 28.6) ²⁰⁶. However, epidemiological data are conflicting. In the Patent Foramen Ovale in Cryptogenic Stroke (PICSS) substudy of WARSS, PFO was identified in 34% of patients who agreed to undergo transoesophageal echocardiography ²⁰⁷. 2-year event rates for recurrent stroke were 14.8% in patients with PFO and 15.4% without PFO (HR 0.96, p=0.84). PFO size and presence of atrial septal aneurysm were not related to outcome.

Optimal medical management of patients with PFO is uncertain. In the WARSS PICSS substudy, no difference in 2-year stroke recurrence was seen, in patients with PFO, between ASA (13.2%) and warfarin (16.5%) therapy, HR 1.17, p=0.65 ²⁰⁷.

PFO closure devices were studied extensively in non-randomised comparisons with medical therapy, with suggestion of superiority of closure ²⁰⁸. RCTs have subsequently indicated a trend toward reduced stroke rates with closure compared with medical therapy but at the expense of higher rates of AF ²⁰⁹.

1.4.13.2. Left ventricular systolic dysfunction

Approximately 10% of patients with prior ischaemic stroke have an impaired left ventricular ejection fraction (LVEF) of $\leq 30\%$ ²¹⁰. LV thrombus formation is common in such patients ²¹¹ and anticoagulation may be preferable to antiplatelet based secondary prevention in such patients, even in the absence of other specific indications for anticoagulation ¹⁹. The WARCEF study, evaluating stroke risk according to antithrombotic regime is ongoing ²¹².

1.4.13.3. Mural thrombus

Acute MI is a common complication following stroke or TIA. Similarly stroke and TIA frequently occur following acute MI. Intracardiac thrombus commonly develops following acute MI (approximately one third of patients with untreated anterior MI and a higher proportion of patients with large infarcts involving the LV apex ²¹¹. Cerebral embolism has been reported to occur in 8% of such patients ²¹³. Three trials comparing anticoagulation (initially with heparin, followed by warfarin) with placebo, demonstrated a reduction in embolism from 3% to 1% ²¹⁴. American Heart Association (AHA) & American Stroke Association guidelines recommend that acute MI complicated by LV mural thrombus, demonstrable on cardiac imaging, should be treated with oral anticoagulation (target INR of 2 – 3) for a period of 3 months (after which the risk of embolism declines) ²¹⁴.

1.5. Summary & aims of this thesis

Approximately half of patients in contemporary acute stroke studies still experience an unfavourable outcome (death or dependency) at 90 days. The risk of stroke and other cardiovascular events following an index stroke or TIA is very high. Changing population demography is likely to result in an increasing burden of cerebrovascular disease. Accordingly, to reduce this burden, secondary preventative strategies must be optimised and potential novel therapeutic strategies evaluated. This thesis addressed aspects of secondary prevention in each of these regards. Firstly, optimised detection (and thereby treatment) of atrial fibrillation after ischaemic stroke and TIA. Secondly, evaluation of a potential therapeutic strategy for the prevention of cardiovascular disease in the stroke population: xanthine oxidase inhibition (XOI).

The potential clinical relevance of enhanced AF detection immediately following stroke is reviewed in Chapter 2. Guidance regarding investigation for PAF following ischaemic stroke and TIA lacks a robust evidence base. Chapter 3 details a randomised clinical trial, which aimed to provide a randomised evidence base to guide clinicians managing unselected ischaemic stroke & TIA patients, in the investigative approach for optimal detection of AF. A related observational study aimed to evaluate the positive and negative predictive value of AF detection in the days following ischaemic stroke, for presence of AF after 90 days follow up from the index event.

In Chapter 4, the potential role of uric acid and XO enzymatic activity in the pathogenesis and pathophysiology of cardiovascular disease is reviewed. In Chapter 5, the available evidence for XOI, in relation to the management of cardiovascular disease, is examined in a systematic review and meta-analysis. Chapter 6 reviews the utility of carotid intima media thickness, BP pulse wave analysis and endothelial function assessment, as both vascular biomarkers and surrogate measurements for modified cardiovascular risk in clinical trials. In Chapter 7, a double blind randomised controlled trial, evaluating the XOI Allopurinol in a population with recent ischaemic stroke or TIA, is reported. This study sought to provide evidence relating to sustained treatment effects of XOI in the ischaemic stroke population, in relation to surrogate markers of CV health, including carotid intima media thickness and central BP.

Chapter 2

The role of atrial fibrillation in the aetiology of ischaemic stroke

2.1. The epidemiology of AF

Atrial fibrillation is the commonest cardiac dysrhythmia ¹⁹⁷. Incidence increases with age, rising exponentially from 0.64, in males < 55 years, to 40.06 per 1000 person-years, in males ≥ 85 years ²¹⁵. The incidence is higher in males than females. In addition to increasing age, AF is observed more frequently in individuals with CVRFs and established cardiovascular disease, including cardiomyopathy, LVH, hypertension, obesity, diabetes, respiratory failure and alcohol excess ^{216, 217}.

The age-adjusted incidence of AF has reportedly increased in recent decades, from 3.04 (per 1000 person-years) in 1980 to 3.68 in 2000 ²¹⁵. This may reflect greater likelihood of detection due to increased availability of more extensive and sensitive screening investigations in recent years and highlights the potential for underestimation of the true prevalence of the condition due to underdiagnosis of patients with clinically silent or PAF ²¹⁸.

Several large population studies have estimated the prevalence of AF. Estimates vary, in part reflecting differences in population characteristics but also variation in the modalities, availability & intensity of investigative strategies for AF between studies ²¹⁹.

Methodology to identify cases of AF have included biennial ECG screening and case record reviews in the Framingham population ¹³⁴, case record review in the Rochester population ²²⁰ and a single screening ECG followed by patient self-reporting to investigators in the Cardiovascular Health Study ²²¹. Irrespective of variability in estimates between studies, as a chronic condition, with age related increasing incidence, the prevalence of AF increases dramatically with increasing age ²²². In these studies, AF prevalence was estimated at 5.9% in individuals aged over 65 years. In the ATRIA study, conducted in a large Californian population, the overall adult population prevalence AF was reported to be 0.95% (95% CI, 0.94 – 0.95%). However, prevalence varied considerably with age such that only 0.1% of individuals < 55 years in age had AF compared with 9.0% of individuals aged ≥ 80 years ²²³. The prevalence quoted in this study excluded patients with transient episodes of AF. All of these studies have the potential to underestimate the prevalence of PAF, as they did not routinely utilize prolonged cardiac monitoring techniques. Those studies which not utilise

any ECG screening (e.g. ATRIA, Rochester) are likely to have underestimated the prevalence of asymptomatic AF of any type.

Population demography has changed in recent years, with the elderly population both increasing in number and as a proportion of the population as a whole, a trend that is expected to continue ¹⁰. This has health and economic implications for chronic conditions and those related to aging, such as AF and in turn, stroke.

Based on the observed prevalence of AF in the ATRIA study, and anticipated changes in population demography, the number of adults in the USA with AF is predicted to increase from approximately 2.3 million in 2000, to 5.6 million by 2050 ²²³. More than 50% of these patients would be over 80 years in age.

2.2. Pathophysiology and natural history of AF

Aetiological factors for the development of AF include ischaemia (e.g. CHD), increased atrial pressure & distension (e.g. chronic heart failure (CHF), valvular heart disease and left ventricular hypertrophy ²²⁴), fibrosis (e.g. ageing, following MI), inflammatory, infective & infiltrative processes ²¹⁸. Risk factors for development of AF include age, male sex, cardiac and other vascular conditions ^{223, 225}. In addition, a positive family history appears to confer increased risk ²²⁶ and several genetic factors have been associated with the condition ²²⁷⁻²²⁹.

The development of electrical re-entry circuits within the atria are a key step in the pathogenesis of AF. The pulmonary veins, at the site of entry to the left atrium have been identified as a potential initial ectopic focus for the development of AF ²³⁰. Aetiological factors for AF frequently predispose to progressive or permanent structural and electrophysiological myocardial remodelling. AF is itself associated with such remodelling, which in turn predispose to recurrence, or maintenance of the dysrhythmia. This has led to the observation that “AF begets AF”; once a threshold for AF to develop has been reached, remodelling confers an increased propensity to recurrence ²¹⁸.

The natural history of AF is now considered a progression between paroxysmal, persistent and permanent phases of the dysrhythmia ¹⁹⁷. Paroxysmal AF describes episodes of AF of brief duration, with spontaneous cardioversion to sinus rhythm. Persistent AF refers to episodes of AF of at least 7 days duration. Cardioversion to sinus rhythm may be successful, albeit only in the short term. Permanent AF describes patients found to be in AF at all times and in whom attempts at cardioversion to sinus rhythm are unsuccessful. Progression through these phases may occur over a variable period of time according to individual patient characteristics, though only a minority of patients will fail to progress, usually in the absence of any other CV disorders ²³¹. Comorbidities and age accelerate the progression of AF & development of complications ^{232, 233}. Progression may be amenable to pharmacological or other intervention in suitable patients ¹⁹⁷.

Several modifications in function of the atria predispose to the development of atrial thrombus in AF, which in turn forms the mechanistic basis for thromboembolic complications of the dysrhythmia. These include stasis of blood, particularly within the left auricular appendage ²¹⁸. Additionally, denuded and dysfunctional endothelium, low grade vascular inflammation and disordered coagulation and platelet activity are all considered to contribute to thrombogenesis in AF ²³⁴.

2.3. Morbidity and mortality related to AF

AF typically presents as one of two clinical syndromes. The first is a symptomatic presentation, which may be typically indicative of the dysrhythmia (e.g. palpitations), non-specific (e.g. lethargy, fatigue, reduced exercise capacity, dyspnoea) or haemodynamic (e.g. pre-syncope). The second clinical presentation is with thrombo-embolic complication, typically ischaemic stroke, though embolic events to non-cerebral arterial territories may also occur. A third clinical presentation, with a tachy-cardiomyopathy, related to persistently elevated ventricular rate and decompensation in cardiac function, is less frequently observed.

AF appears to be an independent predictor of mortality²³⁵. In the Framingham population, presence of AF was associated with an OR for death of 1.5 (95% CI, 1.2 to 1.8) in men and 1.9 (95% CI, 1.5 to 2.2) in women, after adjustment for age and CVRFs. AF doubled the risk of mortality in patients without any history of CV disease and eliminated the survival advantage females hold over males when present.

2.4. AF as a risk factor for stroke

Patients with AF are at increased risk of ischaemic stroke compared to individuals without AF, with several population studies identifying AF to be an independent risk factor for stroke. In the Framingham population, AF independently increased the risk of stroke fivefold ($p < 0.001$)¹³⁴. In this population, AF was the only risk factor for which the attributable risk of stroke increased with age, rising from 1.5% for those aged 50 – 59 years to 23.5% for those aged 80-89 years.

AF has traditionally been considered to account for one fifth of cases of ischaemic stroke^{124, 236}. However, the risk associated with AF increases with age, and in more elderly individuals, AF accounts for a greater proportion of stroke cases as the aetiological factor¹³³. Risk of recurrent stroke is also increased amongst those with AF at the time of presentation with index stroke compared to those without evidence of AF^{133, 237}.

Beyond the increase in risk, stroke severity may also be increased when attributable to AF²³⁸. Data from the Lausanne Stroke Registry reported that AF-related strokes are more often severe and are associated with poorer prognosis^{239, 240}. Higher levels of disability have been reported in long term follow up in AF related stroke²⁴¹ and cardioembolic stroke is associated with higher mortality rates than other TOAST categories at both 30 days and 5 year follow up¹¹⁷. In-patient costs for stroke patients with AF exceed those without²⁴². However, evidence from the VISTA / SITS thrombolysis trial registries suggest that outcomes in rt-PA treated AF patients do not differ significantly from those without AF²⁴³.

Beyond an increase in symptomatic morbidity, the principal clinical relevance of the anticipated increased population prevalence of AF will be an increase in the number of

patients suffering ischaemic stroke and other systemic embolic events as a complication²²³. Given that the increased AF prevalence is due to an expanding elderly population, the increase in stroke may be disproportionately large for the increased number of AF cases. In 2000, approximately 36.7% of patients with AF were aged ≥ 80 years but this is set to increase to increase to 52.6% of AF patients by 2050.

2.5. Risk stratification in patients with AF

Identification of AF and implementation of optimal secondary preventative strategies, particularly in patient subgroups with the highest absolute risk (e.g. following ischaemic stroke & TIA or in the presence of other risk factors), will be a crucial component of the strategy to limit the anticipated burden of cerebrovascular disease.

2.5.1. Risk stratified according to concomitant risk factors

Epidemiological studies examining the relationship between AF and risk of subsequent stroke have identified several co-risk factors which independently increase the risk of an event in the setting of AF¹³⁴.

The Framingham study authors examined RR according to age. Though AF was a significant predictor of risk across all age groups, the strongest association was observed amongst the most elderly. Following adjustment for other risk factors, the influence of age in AF-related stroke risk appeared to be an independent effect¹³⁴. In patients with prior ischaemic stroke / TIA, the risk of stroke / SE / major bleeding / ICH & mortality is increased a further two to threefold, compared with individuals without such prior history^{135, 136}. These observations have permitted the development of risk stratification tools which permit individualised estimation of risk. Large population studies have provided the means to evaluate the relative contribution of such factors to increased risk, ascertaining an independent contribution and combining these in risk prediction models¹³⁶.

2.5.2. Risk according to subtype of AF

There has been debate as to whether different subtypes of AF confer similar risk of stroke, independently of concomitant risk factor burden. Available data are conflicting, with suggestions that paroxysmal AF may confer variously less, equal or greater risk than persistent and permanent forms.

The Euro Heart Survey reported similar risk of stroke for paroxysmal AF as for persistent (OR 0.68; 95% CI, 0.32 – 1.46) or permanent AF (OR 0.69; 95% CI 0.34 – 1.38), on multivariate analysis^{244, 245}. Analysis of the SPAF clinical trial research programme population data also suggested that paroxysmal AF confers similar risk to permanent AF, with annualised stroke rates of 3.2% and 3.3%, respectively²⁴⁶. Similarly, in the ACTIVE-W study, the annualized risk of stroke or SE was 2.0% in PAF patients compared with 2.2% in sustained AF [RR (adjusted for confounding baseline variables) 0.94; 95% CI, 0.63 to 1.40].

Conversely, analysis of stroke rates in the SPORTIF clinical trials programme suggested that stroke and SE annual event rates were higher (1.73%) in persistent AF than in paroxysmal AF (0.93%), HR 1.87; 95% CI 1.03 – 3.36²⁴⁷. The ARISTOTLE study reported a modest but statistically significant, lower stroke & SE event rate in association with PAF compared with either persistent or permanent AF [HR (adjusted) 0.70; 95% CI, 0.51 – 0.93]²⁴⁸. The authors concluded that previous analyses which failed to identify differential risk between AF subtypes may have lacked sufficient statistical power for this. However, the RE-LY study, which included a similar number of patients to ARISTOTLE, reported similar event rates between AF subtypes during a mean follow-up period of 2 years, with paroxysmal, persistent and permanent AF groups experiencing annual event rates of 1.32%, 1.55% and 1.49% respectively²⁴⁹. The reasons underlying the differing findings between the RE-LY and ARISTOTLE trials are open to speculation. The ROCKET-AF study may provide additional information in this regard.

If PAF and non-paroxysmal AF confer differential risk of stroke and embolism, the difference appears likely to be small. Moreover, the available evidence suggests that PAF patients benefit from anticoagulation similarly to those with more sustained forms. The EAFT clinical trial, which demonstrated benefit of anticoagulation in patients with AF, enrolled patients

with both paroxysmal and chronic AF, with approximately three quarters of patients in the latter category ¹³⁷. No differential treatment effect between these AF subgroups was reported. In the ACTIVE-W study, no difference was observed in the efficacy of either anticoagulant (RR (adjusted for other risk factors) 1.14; 95% CI 0.59 – 2.20), or antiplatelet (RR (adjusted for other risk factors) 0.84; 95% CI 0.51 – 1.40) therapy in reducing stroke & SE for PAF compared with sustained AF ²⁵⁰. Similarly, trials comparing NOAC agents with adjusted dose warfarin have reported no differential treatment effect according to the AF subtype ^{248, 249}. In the RE-LY study, lower dose Dabigatran was associated with significantly greater reduction in stroke & SE in PAF patients, compared with both persistent and permanent AF patients (p for interaction = 0.05). The authors concluded that this finding may have reflected play of chance.

Clinical guidelines have made recommendations for the management of AF patients' thromboembolic risk according to the presence of additional risk factors for stroke, rather than on the basis of the subtype of AF (or on the basis of further stratification of the burden of AF in the subgroup with paroxysmal AF) ¹⁹⁷.

2.5.3. Risk according to AF burden

Beyond the debate regarding whether paroxysmal AF confers similar risk to persistent and permanent AF, a secondary question has arisen as to whether all patients with evidence of paroxysmal AF share a similar risk. An extrapolation of the findings in the ARISTOTLE population that AF subtype influences risk, is that the relationship between the overall burden of AF and embolic risk may represent a continuum. If a threshold AF burden exists below which the associated risk is either negligible or insufficiently elevated to justify anticoagulant therapy, this potentially holds clinical relevance ²⁵¹. This is particularly the case given that detection of brief and isolated episodes of AF has been enhanced with the emergence and availability of prolonged cardiac monitoring techniques ²⁵². Several groups have attempted to further refine risk stratification within the paroxysmal AF group.

The MOST study was a large RCT comparing DDDR versus VVIR pacing in sinus node dysfunction patients ²⁵³. In a subgroup of 312 patients, PPMs were programmed to log atrial

high rate episodes (AHREs). In Cox regression, the presence of any AHRE > 5 minutes duration (observed in 160 patients) independently predicted subsequent risk of total mortality, death, non-fatal stroke and diagnosis of AF.

Subsequently, in the TRENDS study cohort, the risk of subsequent vascular events in 2486 patients receiving pacemakers or ICDs was examined according to the burden of atrial tachyarrhythmia (AT) / AF detected by the device. In the 24% of patients with any AT / AF episodes (of ≥ 20 seconds), the median daily burden was 5.5 hours. The annualised TE rate was 1.1% for patients with zero AT / AF burden. Compared with zero burden, patients with “low” AF / AT burden (< 5.5 hours) were at similar risk (HR 0.98; 95% CI, 0.34 – 2.82) whereas those with “high” burden of AF / AT episodes were at increased risk (HR 2.20; 95% CI, 0.96 – 5.05)²⁵⁴. The authors concluded that embolic risk is a quantitative function of AT / AF burden.

In the ASSERT study population, amongst 2580 patients with a PPM or ICD, without prior history of AF, subclinical atrial tachyarrhythmia episodes (> 6 minutes duration) were detected in 10.1% of patients within 3 months. These episodes predicted development of clinical AF (HR 5.56; 95% CI, 3.78 - 8.17) and of ischaemic stroke / SE (HR 2.49; 95% CI, 1.28 – 4.85), compared to patients without any episodes²⁵⁵. Compared with patients without any episodes, the risk of stroke was similarly increased in patients with AT episodes, irrespective of episode duration: > 6 minutes (HR 1.76; 95% CI, 0.99 to 3.11; P=0.05); > 6 hours (HR 2.00; 95% CI, 1.13 to 3.55, p=0.02); > 24 hours (HR 1.98; 95% CI, 1.11 to 3.51; P=0.02).

In 725 patients with PPMs for bradycardia, device detected AF episodes were stratified as either absent, of ≥ 5 minutes duration or ≥ 1 day duration. Following adjustment for other risk factors, only patients with episodes ≥ 1 day duration were associated with increased likelihood of embolic events (OR 3.1; 95% CI, 1.1 to 10.5, p = 0.044), whilst episodes ≥ 5 minutes were not associated with increased risk (though statistical power was limited by a small number of observed events)²⁵⁶.

These studies varied considerably in the observed burden which appeared associated with differential risk. Moreover, it is important to note that AHREs and atrial tachycardias were not characterised further i.e. it is unclear what proportion of these represented AF. Of the

studies to have examined risk in relation to dysrhythmia burden, the briefest duration which constituted an event was 20 seconds (in the TRENDS study). Accordingly there is limited data relating to the relevance of briefer episodes, except that which may be inferred from the above studies. Thus, atrial high rate episodes and ATs of sustained duration may be associated with an increased risk of CV events compared with briefer episodes but definitive conclusions relating burden of AF with risk cannot yet be drawn.

2.6. Addressing the risk of recurrent stroke in patients with AF

As discussed in chapter 1.3.6.4, the risk of recurrent stroke is elevated in patients with AF. The risk of early stroke recurrence is high and remains so in the longer term. On this basis, there is potential to modify risk with intervention on both an immediate and delayed basis. The merits of antiplatelet and anticoagulant therapy in this regard were discussed in chapter 1.2.4 & 1.4.8 and 1.2.5 & 1.4.9, respectively. Whilst both strategies confer benefit, there remains some debate regarding which of these strategies represents the optimal initial acute intervention and regarding the optimal timing for initiation of anticoagulant therapy.

The principal barrier to early use anticoagulation following ischaemic stroke is the risk of early haemorrhagic transformation of cerebral infarcts^{91, 130, 131}. This complication typically arises between 1 and 4 days following stroke onset and rates of haemorrhage appear to be increased in the context of antithrombotic therapy^{125, 131}. This has led to concern that such agents should be avoided in the period immediately following acute stroke. Despite these concerns, the potential for greater reduction in early recurrence in AF patients through anticoagulation has been examined in several clinical trials, including the IST and HAEST.

The largest study to evaluate therapeutic anticoagulation in this setting was the IST. Patients were treated with subcutaneous UFH. Median time to treatment was approximately 19 hours and this continued for 14 days. On the basis that observed increased rates of bleeding (s-ICH, major extracranial haemorrhage) with UFH treatment negated the significantly reduced ischaemic stroke recurrence, the authors concluded that heparin should not be routinely used in acute stroke, including in the subgroup of patients with AF, in whom high

dose UFH was associated with a haemorrhagic stroke rate of 2.8% compared with 0.4% without UFH therapy, at 14 days^{53, 56}.

Methodological limitations included lack of therapeutic monitoring, a lack of subgroups analysis based around concomitant use of ASA, open treatment allocation, which may have favoured investigation for haemorrhagic complication in anticoagulated group, and approximately one third of patients being randomised and treated prior to exclusion of ICH with CT imaging. The proportion of these patients with ICH evident on initial imaging was not reported and how ICH in such patients was distinguished from a new ICH was not referred to in the methodology. Assuming a 15% haemorrhage rate in those without CT, it is feasible that the study lacked sufficient power to identify an overall treatment benefit with anticoagulation.

In the HAEST study, 449 AF patients with ischaemic stroke were randomised to 14 days treatment with IV LMWH or ASA⁵⁸. Average time to randomised treatment was 20 hours. No difference in recurrent ischaemic stroke, ICH events or functional outcomes was noted between treatment groups. However, an excess in a composite of adverse clinical events was observed with Dalteparin. As in the IST, patients in HAEST were not excluded on basis of size of cerebral infarct and outcomes were not stratified on the basis of imaging infarct topography. In addition the population was a relatively elderly one and the severity of stroke in this population was relatively high; 65% of patients had an outcome of death or dependency at 90 days. In addition, SBP was high in the study population overall and significantly higher in the LMWH group compared with ASA. Each of these factors might be considered to increase the hazard of anticoagulation. However, routine use of treatment dose LMWH cannot be recommended in acute stroke with AF on the basis of this study.

A meta-analysis of anticoagulation in acute stroke patients with AF concluded no overall benefit, noting that selected subgroups, which conceptually might be more likely to benefit, have not been studied¹³². No other studies of similar size to the IST have evaluated anticoagulation in acute stroke. Consequently, guidelines are principally based on the data from the IST and the maxim that anticoagulation has no “routine” role in the management of acute ischaemic stroke has propagated since.

There is also a paucity of evidence regarding use of parenteral or immediate anticoagulation in patients with TIA. Although TIA has historically implied a lack of infarct burden due to only transient arterial occlusion and cerebral ischaemia, recent studies with MRI based imaging have demonstrated that a high proportion of patients with clinical TIA exhibit evidence of cerebral infarction¹⁰⁷. It may be inappropriate to assume negligible risk of anticoagulation in this population, who equally may have the most to gain from prompt treatment.

Although there is methodologically limited evidence regarding the use of parenteral anticoagulation in AF patients during the first 14 days following ischaemic stroke, secondary prevention studies of OAC agents provide a robust evidence base for treatment commenced up to 6 months following ischaemic stroke. In addition, they provide some data supporting early implementation of oral anticoagulation from within 2 weeks of ischaemic stroke. Induction of treatment following greater delays and indefinite treatment is recommended on the basis that survival curves continued to diverge at the end of study follow up in the EAFT¹³⁷.

In patients with mild severity ischaemic stroke or TIA, oral anticoagulation with VKAs from 2 weeks after stroke and TIA appears beneficial. Approximately 40% of patients in the EAFT were enrolled prior to this. Though a subgroup analysis according to randomisation time from ictus was not presented, this provides indirect evidence supporting earlier institution of treatment in this population.

There is limited evidence for commencing NOACs prior to 14 days subsequent to stroke, with the major trials enrolling only a small % of patients prior to 14 days and no patients within the first 7 days after stroke. The subgroup of patients in ARISTOTLE with prior ischaemic stroke or TIA were randomised to treatment only after a minimum of 7 days of their qualifying event. 6.8% of those in this subgroup were randomised with an event < 30 days prior. Patients in this group had non-significantly higher stroke & SE rates than those recruited > 30 days (3.71 vs. 2.80 per 100 years follow up, HR 1.31 (0.76 – 2.26). A trend toward greater benefit of Apixaban was noted in patients recruited with greater proximity to their qualifying event.

In both the RE-LY and ROCKET-AF studies, all stroke patients were excluded with ictus < 14 days and major strokes were excluded with ictus < 6 months & < 3 months, respectively). Considered together with the EAFT, there remains a paucity of data relating to the timing of very early anticoagulation efficacy & safety amongst patients with more severe stroke.

In summary, whilst detection of AF in the period immediately following ischaemic stroke may prompt consideration of immediate anticoagulation to reduce the considerable risk of early recurrence, there is no evidence to definitively support this approach. The available evidence, methodological limitations accepted, suggests that in unselected stroke patients, bleeding complications appear to negate the benefit of immediate parental anticoagulation in reducing ischaemic event recurrence. The available evidence is however limited and there may be subgroups of patients who would benefit, including the TIA population for whom evidence is entirely lacking. Early detection of AF will facilitate prompt introduction of oral anticoagulation in patients with mild ischaemic stroke and TIA without delay. It is less clear how quickly patients with more severe stroke should commence treatment.

2.7. The prevalence of AF in patients presenting with ischaemic stroke & TIA

Based on prior history and the admission ECG, AF is reported to be present in present in approximately 15 - 25% of patients presenting with stroke or TIA. In the OCSP, 18% (95% CI 15 – 21%) of patients with cerebral infarction were found to have AF ¹²⁴. In a population registry in France, 18.7% of patients with ischaemic stroke had their event categorised as cardioembolic in the setting of AF ²⁵⁷. Although prevalence of AF in patients presenting with AF is variably estimated according to the population studied, the standard quoted figure for the entire unselected stroke population is an underestimate of the prevalence in more elderly patients with acute stroke. In the Framingham study, prevalence of AF was observed to be as high as 30.7% in patients aged 80 – 89 years and only 6.5% in patients aged 50 – 59 years ¹³⁴. In the AVAIL registry, which included patients admitted to ASUs with either ischaemic stroke or TIA, AF was present in 11.8% ¹⁸⁷. It appears that AF may be observed less frequently amongst patients presenting with TIA than with ischaemic stroke ²⁵⁸.

The true prevalence of AF in the ischaemic stroke and TIA population is likely to be higher than these figures. PAF is frequently asymptomatic and population studies have typically relied on a single 12-lead ECG at the time of clinical presentation in estimating AF prevalence.

As discussed in section 1.3.2, more than one aetiological factor for stroke is evident in many patients with ischaemic stroke and TIA. Whilst some authors have historically argued that the culprit aetiology for the index event may dictate the optimal secondary prevention in the context of co-existent pathologies ²⁵⁹, current guidelines suggest that in such circumstance, optimisation of treatment for each aetiological factor may be appropriate, reflecting the patient populations included in clinical trials ¹⁹. This approach reflects the secondary prevention evidence for anticoagulant therapy in AF being derived from a population of ischaemic stroke & TIA patients without exclusion of patients with potential alternative etiological factors ^{135, 137}.

Some authors have asserted that early efforts to diagnose AF following stroke are misplaced, due to the observation similar risk of recurrence between those in sinus rhythm and those in AF ¹²⁴. This observation likely reflected the fact that risk in the sinus rhythm group of patients is not uniform, including patients with both high (LAA) and low (SVD) risk aetiological factors. Crucially, as discussed in section 1.3.6.4, patients with AF are at high risk of early and subsequent recurrence, irrespective of comparable risk to any other aetiological group. Moreover, AF detection is crucial for the individual patient, because treatment with anticoagulation in AF confers much greater protection than antiplatelet based secondary prevention. Whilst the optimal timing for introduction of anticoagulation is debated and lacks a sophisticated evidence base, prompt identification of AF permits timely institution of anticoagulant therapy beyond the initial week following ischaemic stroke.

2.8. The prevalence of paroxysmal AF in ischaemic stroke & TIA patients

Although AF may be diagnosed at the time of clinical presentation with a 12-lead ECG or on the basis of antecedent history, an additional proportion of patients exist with initially occult paroxysmal AF. Evidence from the VISTA registry, suggests that 6.9% (95% CI, 6.0 – 8.0%) of

patients presenting in sinus rhythm and without prior history of AF, will be diagnosed as having AF within the subsequent 90 days²⁶⁰. Patients included in this analysis were subject to two routinely repeated 12-lead ECGs within 72 hours of study enrolment and robust follow up of events but additional investigations resulting in AF detection were otherwise initiated at the discretion of treating clinicians. The majority of identified patients were diagnosed after the initial 48 hours of follow up.

The true prevalence of AF in the ischaemic stroke & TIA population remains uncertain due to the presence of a population with initially occult PAF. In most patients, the majority of episodes of PAF are clinically asymptomatic²⁶¹. Therefore it is apparent that routine or targeted, rather than opportunistic, investigation is likely to be required to more completely define the true burden of occult PAF. Though numerous studies have addressed this issue, methodological issues have limited conclusions regarding the optimal detection strategy and precise estimation of the prevalence of PAF in this population. Reported studies have utilised heterogeneous detection strategies, which have varied in several aspects, including AF detection modality, duration of investigation, patient population and study design.

The majority of studies have included both patients with ischaemic stroke and TIA. However, stratification of findings by subgroup has rarely been quoted. There is a paucity of studies to have examined the TIA population in isolation²⁶². Although many studies were conducted on a prospective basis, they did not include consecutive patients, with patient inclusion based on local clinician discretion²⁶²⁻²⁷⁰. Several studies included only patients described as having experienced cryptogenic stroke²⁷⁰⁻²⁷⁵. Thus, there is relatively limited data available relating to the unselected stroke & TIA population. Moreover, most studies lacked a control group to compare strategies^{90, 262, 264-267, 269, 270, 272-280}. Those which have did not use randomisation^{93, 263, 281-283}. One study, including a population with cryptogenic stroke included a control group and randomisation²⁷¹. Several studies have evaluated more than one detection modality^{93, 263, 264, 267, 268, 277, 279, 281, 282}. However, these tended to be performed in sequence or compared modalities of differing monitoring duration^{93, 263, 267, 268, 279, 282}, thus limiting direct comparability.

Detection modalities utilised in studies examining AF prevalence following stroke have included Holter style monitoring^{90, 93, 262-269, 276-282}, cardiac event monitors (either

implantable or non-invasive)^{267, 270, 272, 279}, continuous electrocardiological monitoring (CEM)^{93, 263, 268, 273, 282, 283}, repeated 12-lead ECGs^{267, 277, 281} and telephonic ECG transmission²⁷⁴. In the case of the latter two, intermittent scheduled recordings have been performed and additional recordings have been prompted by patient symptomatology. Various durations of monitoring have been performed, ranging from 24-hours^{90, 93, 262-269, 277, 279, 281, 282}, 48 hours^{268, 282}, 72 hours^{93, 263, 278, 279}, 7 days^{267, 276}, 21 days^{271, 272}, 1 month²⁷⁰ to many months^{272, 275}. Several studies examined extended time-frame monitoring with comparison of detection at different intervals with the same modality^{276, 278} whilst others examined differing duration of investigation performed with different modalities^{93, 263, 267, 268, 279, 282}.

There have been a limited number of prospective observational studies which have evaluated routine use of extended cardiac monitoring in consecutive ischaemic stroke and TIA patients²⁸⁴. Point estimates for AF detection with Holter monitoring in such studies have ranged between 3.8 – 6.1%. The combined estimate for AF detection, based on a total of 588 patients with predominantly 24-hour duration Holter was 4.6% (95% CI, 0 – 12.7%) , although one of the included studies represented data in a cohort of patients investigated at clinician discretion rather than as routine²⁶⁸ and one study included monitoring of duration 72 hours rather than 24²⁷⁸.

Varied duration of Holter monitoring has been compared in several studies. Stahrenberg & colleagues found that monitoring for 7 days more than doubled the number of patients in whom AF was identified²⁷⁶. Another study reported detection of AF in only 1.2% of patients with 24-hours Holter monitoring, rising to 6.1% with monitoring extended to 72-hours²⁷⁸. Higher detection rates have been reported with Holter monitoring in selected patient populations^{264-266, 277}.

Cardiac event monitoring provides the facility for more extended periods of monitoring with reduced reporting time, as software programming retains rhythm disturbances of likely significance. Such monitoring may be performed either non-invasively^{267, 279} or with implantable loop recorders. Each permit more extended monitoring periods and out-patient management.

Barthelemy and colleagues assessed cardiac event monitoring with the non-invasive R-test device in 60 consecutive patients with ischaemic stroke or TIA ²⁷⁹. All patients were sequentially investigated with 12-lead ECG, 24-hour Holter and finally R-TE (median duration 4 days). AF was evident on the admission ECG in 5 patients. Of the remaining 55, AF was detected in 9 patients with R-TE (10.9%) and 6 patients on Holter (5.5%). The authors somewhat inappropriately concluded that amongst patients with detected PAF, this was significantly more often detected with R-TE than Holter. However, detection did not differ significantly between the two modalities when the full sample was considered. Moreover, 3 patients with AF identified through each of Holter and R-TE had AF present at baseline ECG. Accordingly, the most appropriate conclusion should have been that additional monitoring led to detection of AF in an additional 6 patients ($p=ns$), with a non-significant trend to increased detection with R-TE compared with Holter.

In a similar design, Jabaudon and colleagues reported detection of AF with 7-days cardiac event monitoring, performed in 88 patients derived from a cohort of 149 consecutive ischaemic stroke & TIA patients with a median delay of 55 days after the cerebral event. Patients were excluded from monitoring if they had a diagnosis of AF based on initial and sequential investigation with routine admission 12-lead ECG, clinically indicated repeated 12-lead ECGs and a 24-hour Holter. In addition, a proportion of patients declined the additional cardiac event monitoring. AF was detected with the non-invasive R-test device in 5.7% (95% CI 2.1 – 12.9%) of patients, suggesting that routine investigations at the time of admission with stroke, including 24-hour Holter, may be inadequate for the detection of PAF. A limitation in interpreting the results reported in this study was that approximately one fifth of patients in whom AF was identified (by any modality), already had a prior diagnosis of PAF ²⁶⁷.

Prospective studies examining implantable event recorders are ongoing, though have been limited to selected populations ²⁷⁵.

CEM appears to hold potential for detection of PAF in the period immediately following presentation to hospital. However, studies have reported disparate effectiveness in AF detection. Groups which have included automated analysis of telemetry data have reported high diagnostic yield (7.7%), particularly in the case of monitoring extended to 72 hours ⁹³.

Conversely, in studies which adopted a retrospective design examining “real world” detection of AF amongst patients with cardiac telemetry, without automated or offline analysis, the likelihood of AF detection was remote²⁶³.

The level of evidence available in support of any one strategy is limited. No studies have evaluated clinical endpoints in relation to enhanced AF detection following ischaemic stroke & TIA. The efficacy of each investigative modality relative to the others is uncertain. There are few controlled studies which have compared investigative strategies^{93, 263, 271, 281-283}. When studies have utilised a comparative control intervention, several did so with sequential rather than simultaneous investigation or compared modalities of non-equal monitoring duration^{93, 282}. In such cases it is not feasible to conclude whether CEM is superior to Holter or if the results merely reflect 72 hours monitoring being superior to 24 hours.

In addition, lack of blinding could potentially have biased observed results in controlled studies, several of which were retrospective in nature. There have been no randomised comparisons of detection strategies in unselected stroke patients and only a single small RCT in a cryptogenic stroke population²⁷¹.

There have been relatively few prospective studies of consecutive, unselected ischaemic stroke & TIA patients performed. Studies referring to inclusion of consecutive patients tended to perform analysis based only on those patients who received additional investigation for the purposes of AF detection, i.e. a population enriched with patients in whom clinicians appeared to have a higher index of suspicion for the presence of AF. Several studies included only patients described as having suffered cryptogenic stroke, with criteria by which cryptogenic aetiology being assigned varying considerably. Clinical trial registry data regarding AF detection is available but without relation to a specific investigation strategy for the detection of AF.

Accordingly, extrapolation of reported findings to the broader population is challenging and consequently guidelines regarding investigation for occult PAF following ischaemic stroke make only limited recommendations. Despite this, two conclusions may be drawn from the evidence base for PAF detection following stroke: Firstly, occult paroxysmal AF appears to be common in patients without previously known AF or AF at the time of initial presentation

with stroke ²⁸⁴. Secondly, detection rates for paroxysmal AF are enhanced by more prolonged periods of cardiac monitoring, including Holter monitoring ²⁷⁶, CEM ⁹³ & cardiac event monitoring ^{267, 279}.

2.9. The relevance of paroxysmal AF identified following acute stroke

As discussed in section 1.3.2, multiple potential aetiological risk factors may be present in patients presenting with ischaemic stroke & TIA. Initially occult PAF may reflect the underlying aetiological mechanism for the presenting cerebrovascular event. Alternatively, PAF may not have been directly responsible but may confer future risk of cardio-embolic events. A third possibility is that AF observed in the context of acute stroke represents a complication of the stroke itself. Following stroke, sympathetic tone is increased, both as part of the stress response to the neurological insult and as a component of the systemic response to peri-stroke complications such as sepsis ^{124, 285}. Infarct topography has also been implicated in triggering episodes of AF ^{285, 286}.

It has been postulated that in this third situation, observation of AF in the immediate aftermath of stroke may represent transient phenomenon, which confers no additional subsequent risk and which would not be optimally managed with formal anticoagulation in the long term ^{252, 287}. On this basis, PAF demonstrated through cardiac monitoring soon after presentation has been criticised as a potentially flawed basis upon which to base secondary preventative therapy decisions ²⁸⁷.

It is feasible that admission 12-lead ECG evidence of AF at the point of presentation might also represent AF triggered by the above stressors, representing only a transient phenomenon. However, such evidence of AF has provided the basis for the inclusion of the majority of patients enrolled in anticoagulant secondary preventative studies ^{135, 137}. There are data which suggests that patients presenting with stroke in AF are likely to continue to exhibit AF ²⁸⁸. Data are lacking in relation to the predictive value of PAF identification through extended monitoring in the immediate aftermath of stroke for detectable AF at some subsequent time point.

2.10. Current guidelines & clinical practice

Current guidelines regarding investigation for AF following stroke and TIA reflect the limited evidence base described above: the optimal modality, duration and timing (relative to the incident event) of investigative strategies have not been established. Guidelines make only limited and diverse recommendations, based on extrapolation of detection rates in the available uncontrolled or non-randomised assessment of the various modalities & strategies in differing populations.

For unselected patients, the guidelines typically recommend a single 12-lead ECG and initial continuous ECG monitoring^{29, 79, 289}. This is recommended at least in part on the basis of the high prevalence of heart disease in patients with stroke and high rates of potentially dangerous cardiac arrhythmias in the setting of acute stroke. Beyond this, more extensive investigation, with the purpose of identifying aetiological rhythm disturbance, is referred to only with reference to patients without evidence of other aetiological factors²⁹. In such patients, the guidelines make no specific recommendation regarding the investigative strategy in terms of either modality or duration.

Scottish guidelines do not specifically refer to investigation for AF, making reference only to the conduct of an ECG at the time of presentation and subsequent ECG monitoring only as part of physiological monitoring for deviation in heart rate as an alert to stroke related complications⁷⁹.

UK guidelines recommend checking for the presence of AF but offer no specific guidance on how to do this beyond an ECG at the time of clinical presentation²⁷. The need for 3- or 7-day cardiac monitoring is suggested for consideration in relation to patients with no identified cause for their stroke on initial screening. No specific recommendation is offered with regard to either the modality or patient characteristics which would mandate investigation.

European guidelines recommend a 12-lead ECG followed by 24 – 48 hours of continuous ECG monitoring, though no recommendation is made regarding the modality of CEM²⁹. 24-hour Holter monitoring is recommended for patients with no other identified cause of stroke (e.g. presence of LAA). These recommendations are graded as Class 1, Level A and appear based

on the observation that Holter monitoring detects AF in patients identified as having sinus rhythm at presentation ²⁷⁷ and that Holter will detect AF in 4.6% of patients otherwise identified as having sinus rhythm ²⁸⁴. They note that serial 12-lead ECGs may be sufficient to detect AF in an ASU ²⁸¹. The guidelines also refer to the observation that further extension of monitoring, event monitoring & more selective monitoring may enhance detection rates ²⁶⁷. No recommendations are made regarding more prolonged monitoring or additional investigation in the unselected population of patients with evidence of alternate culprit aetiology.

North American guidelines recommend a 12-lead ECG on admission and cardiac monitoring in the first 24 hours following stroke ^{28, 289}. Whilst it is acknowledged that detection of AF may be achieved in this manner ²⁸⁷, monitoring is recommended on the basis of the potential for significant and potentially lethal cardiac disease and arrhythmia ²⁸⁹. The use of 24-hour Holter monitoring or prolonged event-loop recorders is referred to in the detection of occult arrhythmias but no recommendation is made regarding their use ²⁸.

Clinical practice in relation to detection of PAF in patients with ischaemic stroke & TIA has not been subject to robust audit procedures on national or international scales, potentially reflecting the lack of a reference standard which may be derived from the current guidelines. Clinical practice is likely to vary considerably between centres. Contemporary UK practice was recently evaluated through a survey of UK stroke physicians ²⁹⁰. Based on available responses, UK practice appears variable. Some stroke centres may reserve prolonged monitoring for patients with so-called cryptogenic stroke, some regard 24 hours' Holter monitoring as standard and others may restrict full investigation to patients with cortical or multiple territory infarcts. Strikingly, only 25% of UK stroke physicians routinely use any form of prolonged monitoring for AF detection and 40% rely solely on the admission 12-lead ECG.

2.11. Chapter 3 aims & hypothesis

There is a gap in the evidence relating to the optimal strategy by which to investigate for the presence of PAF following ischaemic stroke & TIA. No randomised comparisons of investigative strategies are available in unselected patients. Available evidence has often been derived in selected patient populations, with exclusion of patients who might well have PAF and potentially benefit from detection.

Guideline recommendations are non-specific and limited. The approach to the unselected patient presenting with ischaemic stroke or TIA is non-evidenced and may result in underdetection of the true burden of PAF in this population, denying patients potentially superior secondary preventative therapy with anticoagulation. Data regarding UK practice confirms that robust investigative procedures are not utilised.

Chapter 3 presents the detail and findings of a pragmatic randomised trial, which tested whether the effectiveness of current guideline-based investigation could be improved, through application of a simple addition to routine approaches: The study aimed to determine, in an unselected population of patients with recent ischaemic stroke or TIA, with no ECG evidence nor prior history of AF, whether supplementing standard guideline based investigation with 7 days' cardiac event monitoring would: 1) increase detection of paroxysms of AF of duration that would justify anticoagulation; 2) increase detection of paroxysms of AF of any duration (including brief episodes of uncertain prognostic significance); and 3) increase use of anticoagulation for prophylaxis of thromboembolism. We also wished to evaluate whether stroke clinicians without specialist cardiology training could reliably interpret the supplementary cardiac event monitoring data.

In a related observational study, patients enrolled in the above RCT were invited to undergo interval non-invasive cardiac event monitoring for AF, 3 months after their index event. The aim of this study was to evaluate whether AF, detected in the period immediately following ischaemic stroke or TIA, is predictive of AF detectable beyond the period of greatest physiological stress associated with the acute stroke. This would provide indirect information regarding the appropriateness of basing therapeutic decisions on AF detection in the period immediately following the acute event.

Chapter 3

Optimisation of paroxysmal atrial fibrillation detection and treatment following ischaemic stroke and TIA

3.1. Study 1: Non-invasive cardiac event monitoring to detect AF after ischaemic stroke: A randomised controlled trial

3.1.1. Introduction

Ischaemic stroke is the leading cause of adult disability in the developed world and the 2nd leading cause of mortality. Healthcare related costs are high and effective preventative strategies are crucial in reducing the burden of disease.

AF predisposes to the formation of thrombus within the left atrium and consequently to thromboembolic events, typically ischaemic stroke. AF independently increases the risk of stroke five-fold¹³⁴ and doubles the risk of recurrent stroke¹⁷⁴, the latter irrespective of the aetiological mechanism for the incident stroke. AF-related stroke is associated with increased stroke severity, which may be associated with worse outcome²³⁸. AF is common and prevalence will increase with the ageing population²²³.

Oral anticoagulant therapy dramatically reduces the risk of ischaemic stroke in patients with AF, particularly those with prior stroke or TIA (TIA)¹⁷³ regardless of the underlying aetiological mechanism. Moreover, it is substantially more effective than anti-platelet therapy^{5, 173}. New anticoagulant drugs such as the direct thrombin and factor Xa inhibitors may be yet more effective with reduced bleeding complications^{192, 194}.

Risk of recurrent stroke is similar with both sustained and paroxysmal AF (PAF) and both forms are optimally treated with anticoagulation²⁵⁰. Accordingly, detection of occult PAF after ischaemic stroke is of critical importance to optimise uptake of treatment with oral anticoagulation²⁹¹.

One in five patients with ischaemic stroke or TIA will have a prior history of AF or is revealed to have AF on their initial 12-lead electrocardiogram¹²⁴ but further investigation is needed to detect culprit PAF and occult PAF which confers future risk²⁹². A recent systematic review suggested that PAF will be detected in a further 4.6% through routine application of 24-hour Holter monitoring, the conventional modality for investigation²⁸⁴, though individual studies

have reported widely variable detection rates²⁹². Alternative investigation strategies such as extended duration Holter monitoring, cardiac telemetry and implantable cardiac event monitors may increase detection rates but are labour intensive, expensive and lack a definitive evidence base supporting their routine use²⁵². It is acknowledged that detection rates increase with more prolonged monitoring²⁵².

Though detection with prolonged monitoring has been explored in uncontrolled longitudinal studies and selected populations (e.g. cryptogenic stroke), no randomised comparisons have been reported for specific investigation strategies in unselected ischaemic stroke patients. Clinical guidelines reflect this uncertainty and are diverse in their conclusions regarding investigation. Typically the guidelines refer to repeated 12-lead ECGs or some form of cardiac monitoring for selected patients but generally offer no specific recommendation regarding modality or duration^{19, 27, 29, 79}. Some stroke centres may reserve prolonged monitoring for patients with so-called cryptogenic stroke, some regard 24 hours' Holter monitoring as standard and others may restrict full investigation to patients with cortical or multiple territory infarcts²⁹⁰.

The optimal investigation strategy, including modality, duration of investigation and patient subgroup remains undefined, not only for efficacy in the detection of AF but also cost-effectiveness within healthcare systems. This study was a pragmatic randomised trial, to test whether the effectiveness of current guideline-based investigation could be improved, through application of a simple addition to routine approaches.

Aims

The study aimed to determine, in a population of patients with recent ischaemic stroke or TIA, with no ECG evidence nor prior history of AF, whether supplementing standard guideline based investigation with 7 days' cardiac event monitoring would: 1) increase detection of paroxysms of AF of duration that would justify anticoagulation; 2) increase detection of paroxysms of AF of any duration (including brief episodes of uncertain prognostic significance); and 3) increase use of anticoagulation for prophylaxis of thromboembolism. The study additionally evaluated whether stroke clinicians without specialist cardiology training could reliably interpret the supplementary cardiac event monitoring data.

3.1.2. Methods

Study Design

This study was a randomised controlled trial, comparing standard clinical practice for the detection of AF, against standard practice plus additional cardiac event monitoring.

Study Population

Patients were eligible within 7 days of transient or persistent symptoms of acute ischaemic stroke, provided they had an admission 12-lead ECG demonstrating sinus rhythm and had neither prior history of AF or atrial flutter nor any irreversible contraindication for long term anticoagulation (Table 3-1). Patients or their legally approved proxy gave written informed consent. The study was approved by the Scotland A Research Ethics Committee (reference 09/MRE00/59).

Sample size

An initial pilot phase of 100 patients was conducted to demonstrate feasibility of early seven day monitoring and provide estimates of AF detection rate to verify assumptions relating to differences in AF detection rates and use of anticoagulant therapy. Provisionally, in anticipation of the need for a future definitive trial, a sample size of 5000 patients was calculated to provide 95% power to detect a difference between 3% (control) and 5% (intervention) detection rates for AF and 90% power to detect a difference between 2% (control) and 3.5% (intervention) anticoagulation rates. Upon completion of 14 day and 90-day follow-up of the 100 participants in the pilot phase, interim analyses of the pre-defined primary and secondary endpoints was planned to inform on progression to the larger main study phase.

Randomisation and masking

Enrolled patients were randomised, via an interactive voice randomisation system, to either a control group or intervention study group on a 1:1 basis. Research nursing staff assigned participants to their intervention following randomisation. Investigators and patients were aware of study group allocation. This allowed pragmatic management decisions to be made without delay based on diagnostic information gained through study procedures.

Table 3-1: Principal eligibility criteria

Inclusion Criteria	<ol style="list-style-type: none">1. Ischaemic Stroke (including TIA, where symptoms last less than 24 hours).2. Brain imaging not suggestive of an alternative diagnosis.3. Sinus rhythm on screening ECG4. Consent to participate, from patient or approved proxy5. Randomisation within 7 days of ictus.
Exclusion Criteria	<ol style="list-style-type: none">1. Previously documented atrial fibrillation2. Known durable cardiac source of embolism (e.g. mitral stenosis or left ventricular akinesia), or other absolute indication for indefinite anticoagulation (based on antecedent history prior to qualifying event)3. Existing treatment with long term anticoagulation4. Unlikely to be available for completion of study procedures5. Clinical decision or expressed refusal to consider long term anticoagulation at a future date if cardio-embolism may be diagnosed6. Pre-morbid condition or concomitant disease that would render subsequent secondary prevention of stroke inappropriate7. Cognitive impairment deemed sufficient to compromise capacity to consent or to comply with the protocol.8. Prisoners.

Study intervention, data collection and endpoint determination

Patients were enrolled from two acute stroke services in Glasgow (Glasgow Western Infirmary & Glasgow Royal Infirmary). Before entry, every patient had a 12-lead ECG that confirmed sinus rhythm. Patients in both groups received standard practice (SP) investigation for the detection of AF, as individually determined at the discretion of the local treating clinical team, consistent with existing guidelines and with national practice^{19, 27, 29, 79, 290}. Investigations that afforded the opportunity for AF detection comprised additional 12-lead ECGs (subsequent to the admission 12-lead ECG), 24-hour Holter monitoring and echocardiography (which, as coupled with cardiac rhythm monitoring, afforded the opportunity for AF detection). 24-hour Holter recordings were reported centrally at the recruiting hospital cardiology laboratory and reviewed thereafter by treating clinicians.

Patients randomised to the intervention group underwent usual SP investigation plus additional monitoring (AM) for the detection of AF (SP-AM). AM comprised 7 days of non-invasive cardiac event monitoring, performed with the Novacor “R-test Evolution 3” device. The device weighs less than 50 grams and garners cardiac rhythm data through two electrodes, placed respectively at the sternum and apex. This approximates to a CM5 lead configuration. The R-test device used a loop recording system to capture cardiac rhythm episodes of 30 seconds duration (the maximum period of dysrhythmia recordable with the R-test device settings employed in the study), triggered automatically by possible AF recognition. Ten seconds of rhythm preceding and 20 seconds subsequent to the trigger point were captured. The device has previously been validated for the purposes of detection of AF detection in relation to 24-hour Holter monitoring^{267, 279}.

Monitoring commenced immediately following randomisation, with interim downloads at 24, 72 and 168 hours to permit interim analysis of any captured events and to avoid “losing” any detected AF episodes (with a 20 minute memory, the device automatically stores the most prolonged rhythm disturbances preferentially over briefer ones). The SP-AM group also had digital 12-lead ECGs recorded at 24 and 72 hours with a Lexor “Cardiolex” electrocardiograph.

The cardiac event monitoring and digital ECG data were transferred to a central cardiac electrophysiology laboratory (Glasgow Royal Infirmary) led by one of the authors (PWM), for storage and analysis. This is an accredited specialist core laboratory, with extensive experience in ECG reporting and cardiac monitoring data for many international trials. A trained technician established whether the recordings were normal or showed possible evidence of AF, based on absence of discernible organized atrial activity and irregular ventricular response. Recordings with suspected AF were reviewed by an experienced electrophysiologist (PWM).

Endpoints

Based on core laboratory interpretation, patients identified as having evidence of AF were subsequently categorised as exhibiting either “sustained” or “non-sustained” paroxysms of AF. “Sustained” PAF was diagnosed where AF was recorded for the complete 20 second rhythm strip following event triggering. This definition accords closely with that recommended for diagnosis within clinical trials²⁹³ and clinical practice²⁹¹ and which would justify consideration of formal anticoagulant therapy. “Non-sustained” PAF included briefer paroxysms of a minimum of 6 conducted ventricular complexes but < 20 seconds’ duration. Such episodes lack a substantial evidence base in terms of prognostic significance and there is clinical uncertainty in terms of benefit of anticoagulant therapy. Hereafter, reference is made to detection of “sustained” PAF and “any duration” PAF (the latter comprising both “sustained” and “non-sustained” PAF patients).

In addition to the external validated reporting of R-test data, local treating clinicians were able to review data captured by the R-test device on a “real time” basis as recordings were collected. Local clinician interpretation of the R-test data in this manner facilitated potential adjustment to patient therapy without delay. Local clinician reports were collated, to permit comparison with the external laboratory reports.

Follow Up

Patients were reviewed at 14 and 90 days, either through a patient visit or, if this was not possible, through telephone discussion with the patient and their primary care practitioner together with review of case notes and relevant investigation reports.

The primary endpoint was the difference in AF detection between patients randomised to receive SP-AM compared with SP alone at 14 days. As above, detection of AF was defined as evidence of “sustained” PAF and “any duration” PAF.

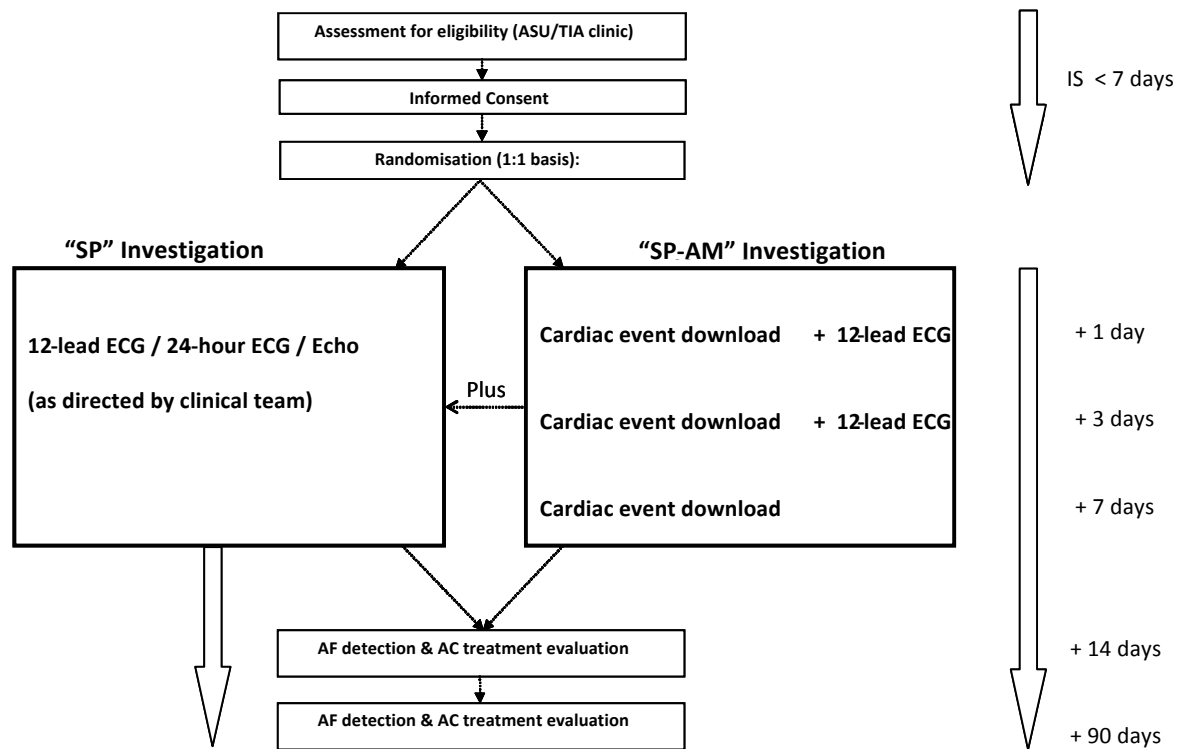
Secondary endpoints were the difference in AF detection at 90 days and the difference in AF-thromboembolic prophylaxis-related anticoagulation (e.g. warfarin) between patients randomised to receive SP-AM compared with SP alone at 14 & 90 days. Data regarding serious adverse events and relevant clinical endpoints (TIA, stroke, MI, death) were also collected to 14 and 90 days, respectively.

The use of other investigations such as trans-thoracic or trans-oesophageal echocardiography and vascular imaging were recorded but their use was not controlled. The study population was neither one of cryptogenic stroke nor enriched according to cortical or multiple territory infarcts. In line with its pragmatic nature, this trial included all patients with sinus rhythm who may eventually benefit from detection of AF.

Statistical analysis

Analysis was performed on an intention to treat basis, with comparison of AF detection (“sustained” and “any duration”) and anticoagulation status by difference in proportions, performed at 14 and 90 days. The Fleiss Kappa statistic was used to assess agreement between local treating clinicians and the validated core laboratory in R-test reporting of AF (Minitab, v 16). Survival analysis, with the log rank test, was performed for each endpoint of interest at 90 days (SPSS, v 18). The study design is summarised in figure 3-1.

Figure 3-1: Summary of Chapter 3, Study 1 design



ASU, acute stroke unit; TIA, transient ischaemic attack; IS, ischaemic stroke, SP, standard practice; SP-AM, standard practice plus additional monitoring; ECG, electrocardiogram; AF, atrial fibrillation; AC, anticoagulation

3.1.3. Results

Interim analysis was performed following 90 day follow up of 100 patients enrolled from two stroke services in Glasgow between May 2010 and September 2011. All 100 patients completed 90 day follow up. There were no significant differences between the randomised groups in either baseline characteristics (Table 3-2) or the investigations performed which comprised standard practice (Table 3-3).

Table 3-2: Chapter 3, Study 1 population baseline characteristics

	Total (n = 100)	SP group (n = 50)	SP-AM group (n = 50)	P value
Age	65.8 (12.3)	64.6 (13.3)	67.1 (11.1)	0.32
Ictus to randomisation, Median (IQR)	1 (1)	1 (2)	1 (1)	0.74 ¹
NIHSS (stroke patients only), Median (IQR)	2 (4)	1 (3.5)	1 (4)	0.82 ¹
SBP	154.8 (27.1)	154.4 (25.3)	155.2 (29.0)	0.88
DBP	81.5 (14.5)	81.5 (14.4)	81.5 (14.7)	1.00
HR	77.55 (17.5)	77.5 (16.2)	77.6 (18.8)	0.96
-OH units / week, Median (IQR)	3 (15)	2.5 (15)	3 (15)	0.71 ¹
Male sex	56%	64%	48%	0.10
Qualifying event Stroke	68%	66%	70%	0.67
Qualifying event TIA	32%	34%	30%	0.67
TACS	14%	14%	14%	1.00
PACS	41%	42%	40%	0.84
LACS	33%	30%	36%	0.52
POCS	12%	14%	10%	0.54
IHD	16%	16%	16%	1.00
Hypertension	58%	60%	56%	0.69
Diabetes	15%	22%	8%	0.09 ²
Smoker	32%	40%	24%	0.08
Aspirin	55%	56%	54%	0.84
Dipyridamole MR	21%	22%	20%	0.81
Clopidogrel	27%	26%	28%	0.82

Any antiplatelet	75%	78%	72%	0.49
ACE-I	37%	38%	36%	0.84
ARB	8%	6%	10%	0.72 ²
Thiazide	18%	14%	22%	0.30
CCB	15%	14%	16%	0.78
Beta-blocker	17%	16%	18%	0.79
Alpha-blocker	2%	0%	4%	0.50 ²
Statin	58%	60%	56%	0.69
Other lipid modifying	2%	4%	0%	0.50 ²
Hypoglycaemic therapy	10%	14%	6%	0.32 ²

Continuous baseline characteristic values are quoted as mean (SD) unless otherwise stated. Comparison was with two-sample t-test except when a variable did not approximate to the Normal distribution, when comparison with the Mann-Whitney test ¹ was used. Categorical baseline characteristics are quoted as %. Comparison between groups is with difference in 2 proportions. When observed frequencies were low, Fisher's exact test ² was used. A p value of < 0.05 was considered statistically significant.

SP, standard practice; SP-AM, standard practice plus additional monitoring; CI, confidence interval; NIHSS, National Institute of Health Stroke Scale; IQR, inter-quartile range; SBP, systolic blood pressure, DBP, diastolic blood pressure; HR, heart rate; -OH, alcohol; TACS, total anterior circulation syndrome; PACS, partial anterior circulation syndrome, LACS = lacunar anterior circulation syndrome; POCS, posterior circulation syndrome; TIA, transient ischaemic attack; IHD, ischaemic heart disease; MR, modified release; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 3-3: Standard practice investigations affording opportunity for AF detection, performed to 14 & 90 days

	SP group (n = 50)	SP-AM group (n = 50)	P value
12-lead ECG (14 days)			
14 days	16% (7.1 – 29.1%)	12% (4.5 – 24.3%)	0.56
90 days	28% (16.2 – 42.5%)	18% (8.6 – 31.4%)	0.23
24-hour ECG monitoring			
14 days	16% (7.1 – 29.1%)	26% (14.6 – 40.3%)	0.22
90 days	60% (45.2 – 73.4%)	46% (31.8 – 60.7%)	0.16
Echo			
14 days	30% (17.9 – 44.6%)	42% (28.2 – 56.8%)	0.21
90 days	62% (47.2 – 75.3%)	54% (39.3 – 68.2%)	0.42
“Any duration” PAF detected			
14 days	4% (0.0 – 13.7%)	12% (4.5 – 24.3%)	0.27 ²
90 days	10% (3.3 – 21.8%)	22% (11.5 – 36.0%)	0.10
“Sustained” PAF detected			
14 days	2% (0.0 – 10.6%)	8% (2.2 – 19.2%)	0.36 ²
90 days	8% (2.2 – 19.2%)	16% (7.2 – 29.1%)	0.36 ²
Values quoted are % (95% CI). Comparison between groups is with difference in 2 proportions. When observed frequencies were low, Fisher’s exact test ² was used. A p value of < 0.05 was considered statistically significant. SP, standard practice; SP-AM, standard practice plus additional monitoring; CI, confidence interval; ECG, electrocardiogram; PAF, paroxysmal atrial fibrillation			

The additional monitoring was well tolerated, with high completion rates and no associated adverse events (Table 3-4). The AM cumulative detection rate for evidence of AF, reported through the core ECG laboratory, was: 4/50 (8%; 95% CI, 2.2 – 19.2%) for the two additional digital 12-lead ECGs; 8/50 (16%; 95% CI, 7.2 – 29.1%) for “sustained” AF with the non-invasive R-test cardiac event monitoring; 21/50 (42%; 95% CI, 28.2 – 56.8%) for “any duration” AF with the non-invasive R-test cardiac event monitoring. Every patient in whom AF was identified by 12-lead ECG at 24 or 72 hours also had “sustained” PAF episodes identified through R-test non-invasive cardiac event monitoring.

**Table 3-4: Additional monitoring (AM) tolerability and PAF detection,
according to individual regime components**

Modality	Completed satisfactorily	“Any duration ” PAF detected	“Sustained” PAF detected
24-hour 12-lead ECG	84%, (70.9 – 92.8%)	8%, (2.2 – 19.2%)	8%, (2.2 – 19.2%)
72-hour 12-lead ECG	82%, (68.6 – 91.4%)	8%, (2.2 – 19.2%)	8%, (2.2 – 19.2%)
24-hour R-test download	94%, (83.5 – 98.7%)	18%, (8.6 – 31.4%)	8%, (2.2 – 19.2%)
72-hour R-test download	90%, (78.1 – 96.7%)	38%, (24.7 – 52.8%)	14% (5.8 – 26.7%)
168-hour R-test download	82%, (68.6 - 91.4%)	42%, (28.2 – 56.8%)	16% (7.2 – 29.1%)
Values quoted are % (95% CI). PAF, paroxysmal atrial fibrillation; CI, confidence interval; ECG, electrocardiogram			

“Sustained” and “any duration” PAF episodes were each detected more frequently in the SP-AM group compared with the SP group at both 14 and 90 days (Table 3-5). 90-day survival free from “sustained” and “any duration” PAF detection are shown in figures 3-2 and 3-3.

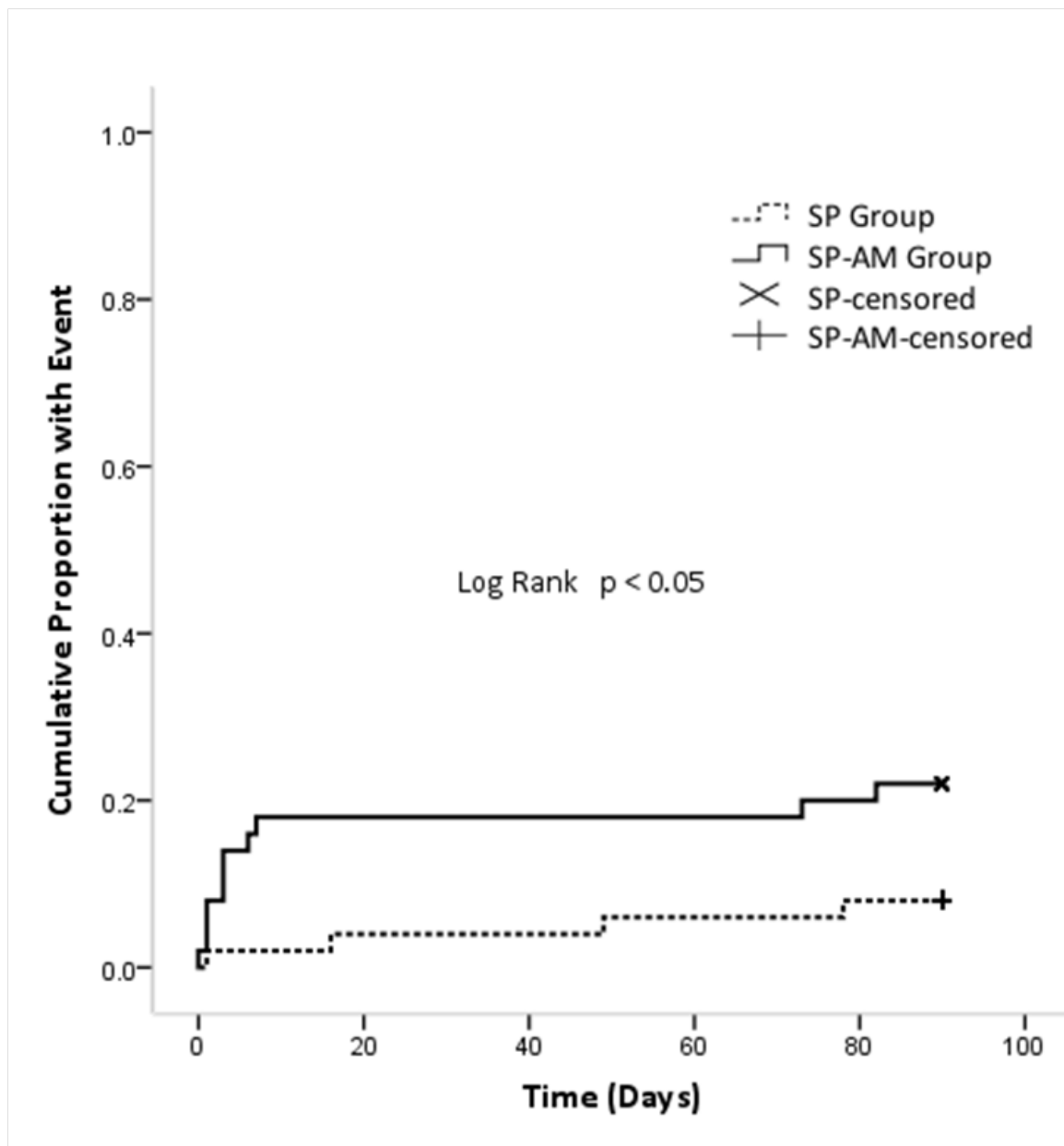
Anticoagulant therapy was initiated more frequently in the SP-AM group compared with the SP group at both 14 and 90 days (Table 3-5). 90-day survival free from anticoagulation for AF-related thromboembolism prophylaxis is shown in figure 3-4. 90-day survival free from anticoagulation for any indication is shown in figure 3-5.

There was excellent agreement between the ECG core laboratory and local clinicians for the presence of “sustained” paroxysms of AF on the R-test event monitors: 48/50 cases (96%, 95% CI 86.3 – 99.5), Fleiss’ Kappa 0.86, $p < 0.00001$. Agreement for the presence of “any duration” AF was present in 44/50 cases (88%, 95% CI 75.7 – 95.5), Fleiss’ Kappa 0.76, $p < 0.00001$. There was no difference between groups for recurrent stroke, TIA, MI, death or any combination of these clinical endpoints (4 combined events in each group). There were no serious adverse events associated with use of the R-test device.

Table 3-5: Differences between groups for detection of “any duration” PAF, “sustained” PAF and treatment with anticoagulation, at 14 and 90 days

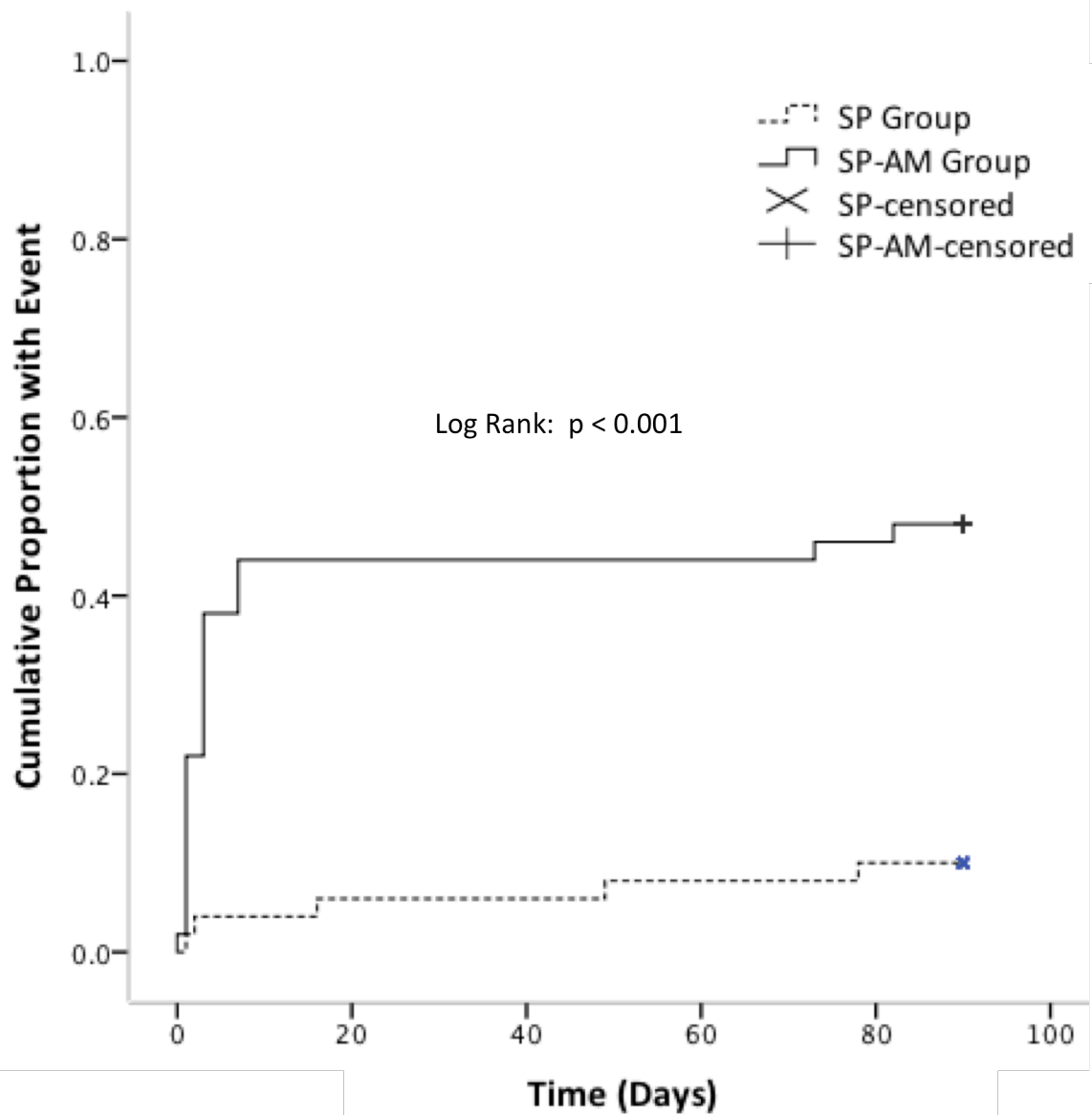
Endpoint	SP Group	SP-AM Group	Difference between groups	P value
“Any duration” PAF 14 days	4% (0.0 – 13.7%)	44% (30.0 – 58.7%)	40% (25.2 – 54.8%)	< 0.001 ²
“Any duration” PAF 90 days	10% (3.3 – 21.8%)	48% (33.7 – 62.6%)	38% (21.8 – 54.1%)	< 0.001
“Sustained” PAF 14 days	2% (0.0 – 10.6%)	18% (8.6 – 31.4%)	16% (4.7 – 27.3%)	< 0.05 ²
“Sustained” PAF 90 days	8% (2.2 – 19.2%)	22% (11.5 – 36.0%)	14% (0.0 – 27.7%)	0.09 ²
AC for any indication 14 days	0% (0 – 5.8%)	18% (8.6 – 31.4%)	18% (7.4 – 28.6%)	< 0.01 ²
AC for any indication 90 days	10% (3.3 – 21.8%)	26% (14.6 – 40.3%)	16% (1.2 – 30.7%)	< 0.05
AC for AF TE prophylaxis 14 days	0% (0 – 5.8%)	16% (7.2 – 29.1%)	16% (5.8 – 26.2%)	< 0.01 ²
AC for AF TE prophylaxis 90 days	6% (1.3 – 16.5%)	22% (11.5 – 36.0%)	16% (2.8 – 29.2%)	< 0.05 ²
Values quoted are % (95% CI). Comparison between groups is with difference in 2 proportions. When observed frequencies were low, Fisher’s exact test ² was used. A p value of < 0.05 was considered statistically significant. SP, standard practice; SP-AM, standard practice plus additional monitoring; CI, confidence interval; PAF, paroxysmal atrial fibrillation; AC, anticoagulation; TE, thromboembolic				

Figure 3-2: Kaplan-Meier curve illustrating detection of “sustained” PAF episodes



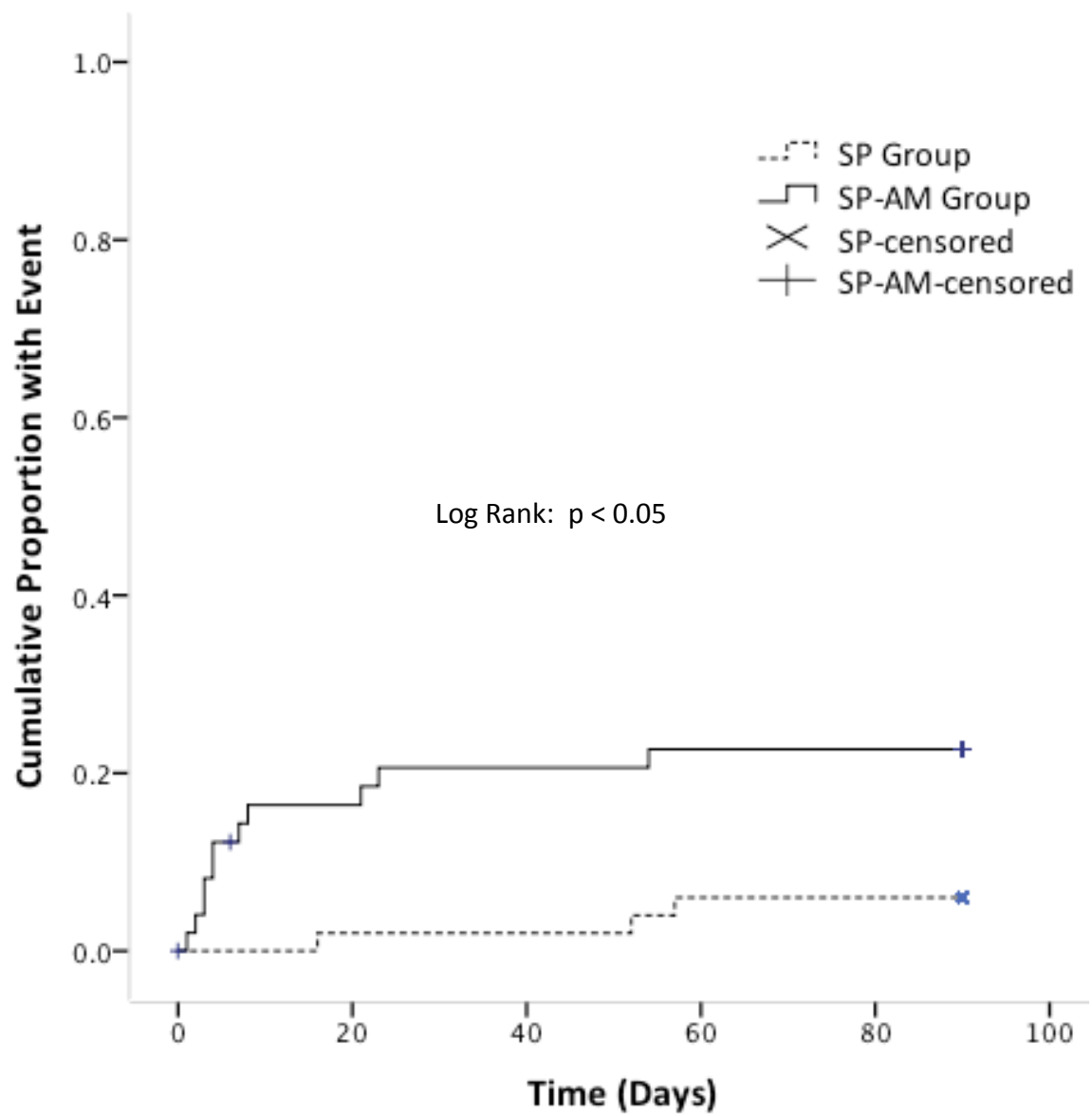
SP, standard practice group; SP-AM, standard practice plus additional monitoring group

Figure 3-3: Kaplan-Meier curve illustrating detection of “any duration” PAF episodes



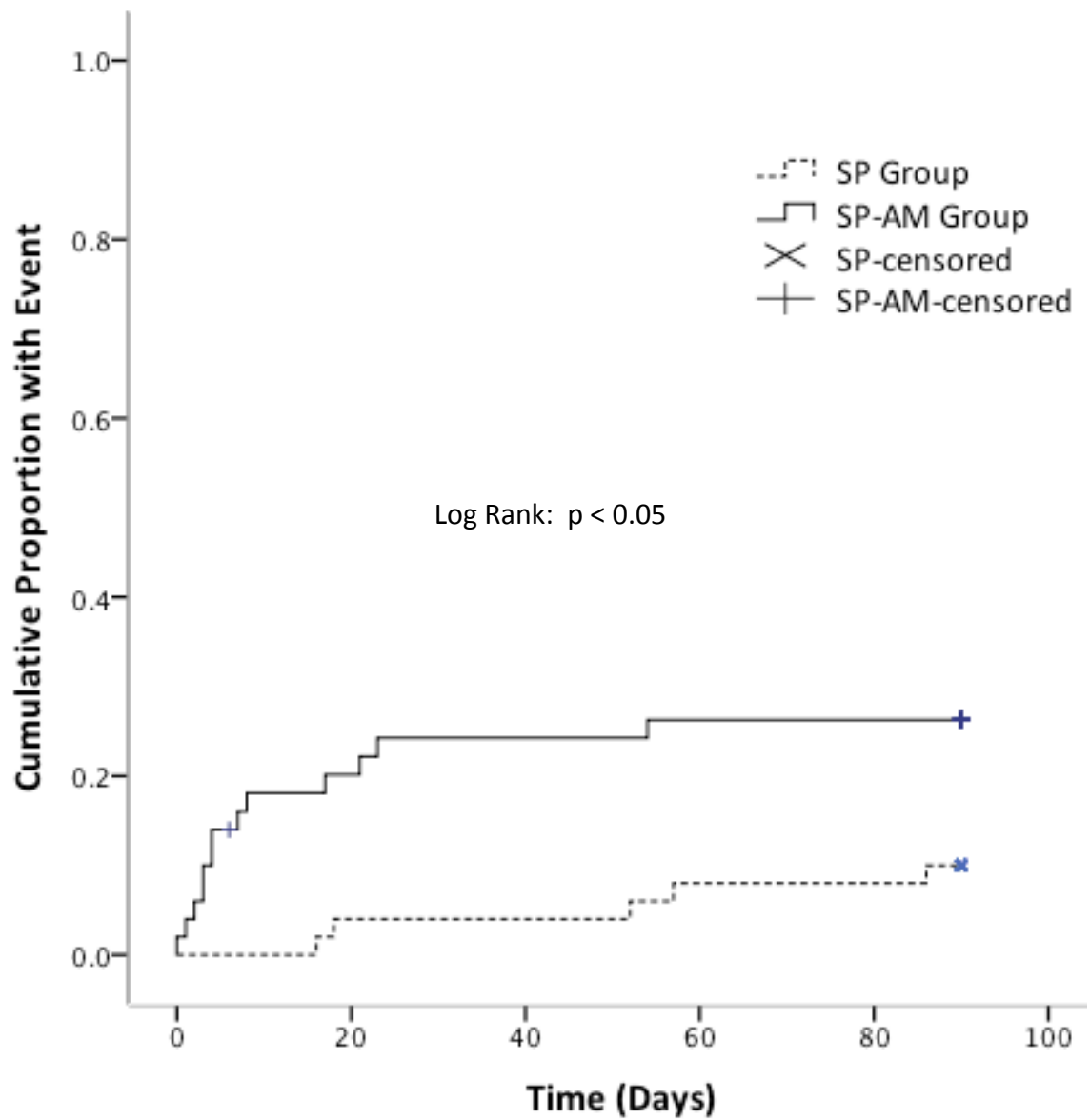
SP, standard practice group; SP-AM, standard practice plus additional monitoring group

Figure 3-4: Kaplan-Meier survival curve illustrating initiation of anticoagulation attributable to PAF detection



SP, standard practice group; SP-AM, standard practice plus additional monitoring group

Figure 3-5: Kaplan-Meier survival curve illustrating initiation of anticoagulation for any indication



SP, standard practice group; SP-AM, standard practice plus additional monitoring group

3.1.4. Discussion

This trial provides the first randomised evidence in an unselected acute ischaemic stroke and TIA patient population comparing investigative strategies for the detection of AF. The study demonstrated superior effectiveness of routine early, extended cardiac event monitoring in all ischaemic stroke patients with sinus rhythm, versus existing guideline-based practice. This strategy also enhanced anticoagulation rates, which should offer improved stroke prevention. Guideline based practice, as currently implemented in the UK & elsewhere²⁹⁰ is inadequate for the prevention of AF-related recurrent stroke and can readily be improved.

Though detection with prolonged monitoring has been explored in uncontrolled longitudinal studies and selected populations (e.g. cryptogenic stroke), no randomised comparisons have previously been reported for specific investigation strategies in unselected ischaemic stroke patients.

Current guidelines reflect the paucity of randomised or controlled evidence evaluating detection strategies, in making limited and diverse recommendations regarding investigation for AF detection following stroke. Typically the guidelines refer to repeated 12-lead ECGs or some form of cardiac monitoring for selected patients but generally offer no specific recommendation regarding modality or duration^{19, 27, 29, 79}. In the UK, no recommendation beyond repeated 12-lead ECGs is made^{27, 79}. International guidelines either disregard prolonged monitoring¹⁹, or recommend its use in selected patients only²⁹, following in-patient continuous electrophysiological monitoring (CEM) of unspecified modality and duration.

Consequently, clinical practice varies widely, with limited investigation for AF in many stroke centres. Some stroke centres may reserve prolonged monitoring for patients with so-called cryptogenic stroke, some regard 24 hours' Holter monitoring as standard and others may restrict full investigation to patients with cortical or multiple territory infarcts²⁹⁰. In the UK, only 25% of stroke physicians routinely use any form of prolonged monitoring for AF detection and 40% rely solely on the admission 12-lead ECG²⁹⁰. Stroke units may lack adequate facilities for routine CEM monitoring and in the absence of automated analysis

software systems, the likelihood of AF detection is remote²⁶³. Out-patient management of TIA and minor stroke patients further limits viability of CEM.

UK standard practice reflects guidelines in utilising Holter devices for extended monitoring²⁹⁰. Standard investigations in this trial reflected wider UK practice: 60% of patients had 24-hour Holter monitoring, especially patients with suspected “embolic” stroke aetiology. The AF detection level of 8% in the SP group was comparable with rates reported in the absence of routine monitoring²⁶⁰ or with 24-hour Holter monitoring²⁸⁴.

Systematic review suggests Holter monitoring will identify AF in only an additional 4.6% of patients²⁸⁴, no better than detection rates observed in groups lacking routine monitoring²⁶⁰. Extending the duration of Holter monitoring should increase detection but consumes more resources²⁷⁶. Without routine use of extended cardiac monitoring following stroke, AF will be detected in only 6 – 8% of patients followed for 90 days²⁶⁰. Extended monitoring increases detection up to 3-fold^{252, 292}.

Detection of AF through additional monitoring in this study compared very favourably against rates reported for 24-hour Holter monitoring²⁸⁴ or repeated 12-lead ECGs²⁸¹ and achieved similar detection rates to extended duration Holter monitoring²⁷⁶, automated software assisted CEM⁹³ and implantable cardiac event recorders²⁷⁵.

Conversely, guideline based practice under-detects AF. Additional monitoring resulted in detection of “sustained” PAF episodes that justify anticoagulation in an additional 16% of patients, within 14 days. This allows initiation of treatment during the period when recurrence risk is highest. Current guidelines suggest initiation of anticoagulant treatment two weeks after stroke onset is safe. As discussed in Chapter 2, there may be patient subgroups who could be safely treated at an earlier stage and a proportion of patients included in secondary prevention anticoagulant trials were enrolled prior to this.

In patients with cryptogenic stroke, in whom simple measures have failed to identify any potential cause, invasive and more costly implantable event recorders with their adherent risks of infection may be justified. They offer greatly extended monitoring periods and high detection rates in some cohorts²⁷⁵. Conversely, a recently reported randomised trial in

patients with cryptogenic stroke recently reported that prolonged monitoring brought no increase in diagnostic yield for AF ²⁷¹.

For the majority of patients, however, this trial suggests that a relatively brief duration of non-invasive monitoring may be sufficient for routine application, with 88% of cases of “sustained” PAF identified within 72 hours. Excellent reporting agreement between treating stroke physicians and the electrocardiology laboratory facilitated immediacy of clinical decisions and could limit costs.

A non-invasive strategy that had few technical failures, no adverse events and which was well tolerated is an attractive option for routine application in unselected acute patients.

Enhanced detection of “sustained” PAF episodes will facilitate delivery of anticoagulant, rather than antiplatelet, based secondary prevention, conferring an additional 6% annual absolute risk reduction ¹⁷⁸ for these patients. The study anticoagulation rate of 75% was high but even using more typical rates of 39% ²³, significant reductions in stroke recurrence would be anticipated due to enhanced AF detection.. The emergence of NOACs may increase the proportion of patients with AF who might be treated with anticoagulant rather than antiplatelet based therapy.

“Any duration” PAF episodes were also detected in 40% more patients receiving additional monitoring. Extrapolating across the UK, this would identify an additional 46,080 patients annually. However, these brief, non-sustained paroxysms of AF are distinct from “sustained” PAF. The risk they confer is uncertain ^{253, 255}, and anticoagulant therapy is of unproven value ³¹. Though permanent and paroxysmal AF share an equal risk of stroke ²⁵⁰, we need a large scale prospective study to evaluate the risk associated with these very brief paroxysms of AF that are now readily detected.

The cost per QALY gained by outpatient monitoring for AF detection is estimated at only [US] \$13,000 ²⁹⁴. This figure excluded indirect savings (e.g. carers' costs) associated with stroke prevention and was based on only a 4.45% increment in AF detection. Additional monitoring, which more rapidly achieved a 16% increment in sustained AF detection, will improve cost-effectiveness.

Limitations

Generalisability of this trial is limited by the sample size and its derivation from only two centres in a single country; however, equipoise was lost after such striking differences were seen at conclusion of the pilot phase. Study patients had relatively mild stroke or TIA but this group potentially has most to gain from expedited AF detection and treatment. The study did not aim to characterise AF detection according to TOAST criteria. This was intentional, as the original randomised trial sought to provide evidence in an unselected ischemic stroke and TIA population, allowing greater generalisation of findings. Detection rates could be higher among patients with extensive cortical stroke, which is indicative of an embolic aetiological mechanism, though many such patients were ineligible because AF was already present.

The study lacked power to examine clinical endpoints as an outcome, though the available evidence suggests that the significant increase in detection of “sustained” episodes of PAF should reduce stroke recurrence through optimised use of anticoagulant therapy. Further study is needed in relation to the significance of PAF episodes of briefer duration.

How readily applicable these data are to non-UK based populations may be debated. The study design did not standardise investigations to any particular investigational strategy in the standard care group, as this does not reflect UK clinical practice nor existing guidelines from the UK, Europe or the USA. All acknowledge that prolonged monitoring may be needed but this is essentially left to physician discretion. Echocardiography or other investigations were not used to screen study patients first for aetiological mechanisms; however, indiscriminate transthoracic and transoesophageal echocardiography offer low returns²⁹⁵ and undirected tests are discouraged in our health service^{29, 79}. The study sought to provide evidence for the approach to unselected patients presenting with ischaemic stroke and TIA rather than for a cryptogenic stroke population.

SP investigations in the centres and across the UK²⁹⁰, likely lie towards one end of a spectrum of guideline interpretation^{19, 27, 29, 79}, across which healthcare funding approaches may exert an influence. Whilst some centres may routinely perform extended monitoring and other additional investigations as routine, funding for health care interventions in many

health care systems is reliant on randomised evidence of effectiveness: this study should allow the level of evidence to be raised and to improve implementation. Whilst the control group design may weaken generalisation to some environments, equally it strengthens applicability to others and we hope that highlighting the deficiency may lead to improvement in guidelines and practice.

It is feasible that the observed difference in AF detection rates between the SP and SP-AM study groups might reflect a greater prevalence of AF in the SP-AM group, arising by chance despite randomisation. It is reassuring to some extent that SP investigations were balanced between treatment groups as was the detection of AF with SP investigations.

Technical limitations of the existing R-test device precluded quantification of the total burden of AF. Improved memory and detection algorithms are now available that could underpin a registry study of stroke recurrence risk in relation to burden of non-sustained AF episodes.

Conclusion

This trial provides randomised evidence that routine extended monitoring for AF in unselected patients with acute ischaemic stroke delivers clinically meaningful and statistically significant improvements in detection and treatment rates versus current guideline based practice. Non-invasive cardiac event monitoring should be routinely adopted as the standard of care in all stroke patients who appear to be in sinus rhythm.

3.2. Study 2: Predictive value of newly detected atrial fibrillation paroxysms in acute ischaemic stroke and TIA, for presence of AF after 90 days

3.2.1. Introduction

AF observed in the context of acute stroke may represent a complication of the stroke itself, attributable to the stress response to the neurological insult and peri-stroke complications such as sepsis ^{124, 285} or in association with involvement of specific areas of cerebral ischaemia ^{285, 286}. If such episodes of AF represent a transient phenomenon only, they may confer no additional subsequent risk and would not be optimally managed with formal anticoagulation in the long term.

Intensive and extended monitoring for PAF immediately following stroke increases detection of PAF episodes of both “sustained” and brief duration ²⁹⁶. Data are lacking in relation to the predictive value of PAF identification through such monitoring, for AF detectable at some subsequent time point, distant from the acute neurological injury. In addition, there are few data relating to the predictive value of PAF episodes of brief duration for more “sustained” episodes on subsequent follow up.

This study sought to evaluate the predictive value of AF detection in the days immediately following ischaemic stroke or TIA, for subsequent AF detection through non-invasive cardiac event monitoring, performed following a 90 day interval from the index event. The predictive value of both “any duration” and “sustained” duration PAF episodes for episodes of corresponding episodes on subsequent monitoring were assessed. In addition, the predictive value of “any duration” PAF episodes for subsequently identified “sustained” duration PAF episodes was assessed.

This would provide information regarding the appropriateness of basing therapeutic decisions on AF detected in the period immediately following the acute event, which has been proposed as a potentially unreliable measure of long term risk ¹²⁴.

3.2.2. Methods

Study Design

This study was an observational substudy extension of the RCT described in section 3.1. All participants were invited to attend for 7 days non-invasive cardiac event monitoring 90 days following their enrolment.

Study Population

Details of the study population, recruitment, cardiac event monitoring methodology and endpoint assessment have been detailed in chapter 3.1.2.

Follow Up

After 90 days, patients who were able to attend for interval R-test monitoring underwent a further 7 day monitoring period. Data from the interval monitoring was downloaded as described in section 3.1.2. Thereafter, captured rhythm strips were reviewed for evidence of AF. Observed episodes were categorised as either “sustained” or “non-sustained” and the combination of either was reported as episodes of “any duration”, as described in section 3.1.2.

Statistical analysis

Baseline characteristics and AF detection at both follow up time points in Study 1 were compared between the group of patients who underwent interval R-test monitoring and those who were unable to, to assess for any differences between these populations.

Comparison of AF detection (“sustained” and “any duration”) between initial investigations and the subsequent interval R-test analysis was performed considering the interval R-test analysis as the “Gold standard” measurement, being performed under stable conditions distant from elevated sympathetic tone and cerebral ischaemia which might potentially influence findings of investigation performed immediately following the index event.

Sensitivity, specificity, negative and positive predictive values (NPV & PPV, respectively), together with 95% CIs, were each calculated for both “sustained” and “any duration” PAF episodes detected by each of: initial R-test alone; initial SP investigation alone (at both 14 and 90 days); combined initial R-test and SP investigation (at both 14 and 90 days); for corresponding “sustained” and “any duration” PAF episodes identified through the interval R-test.

In addition, sensitivity, specificity, NPV & PPV, together with 95% CIs, were each calculated for “any duration” PAF episodes detected by each of: initial R-test alone; initial SP investigation alone (at both 14 and 90 days); combined initial R-test and SP investigation (at both 14 and 90 days); in relation to “sustained” PAF episodes, identified through the interval R-test.

Minitab (version 16) was used to perform statistical analysis.

3.2.3. Results

Interval R-testing was performed in 49 patients (26 patients from the SP group and 23 patients from the SP-AM group). There were no technical failures in the interval R-tests.

Baseline characteristics in patients who participated in this sub study appeared balanced with those who did not. Baseline NIHSS was lower and treatment with ACE-I & calcium channel blocker (CCB) were each higher amongst patients who underwent interval testing. Both “sustained” and “any duration” PAF episode detection were non-significantly higher in the group who underwent interval R-test testing at 90 day follow-up. Table 3-6 includes baseline characteristics and AF detection during study 1 for the population as a whole and the subgroups who did not and did undergo interval R-testing.

Table 3-6: Baseline characteristics and standard practice investigation AF detection rates, stratified according to availability of interval R-test examination

	Total (n = 100)	No interval R- test (N=51)	Interval R-test performed (N=49)	P value
Age	65.8 (12.3)	65.0 (14.7)	66.73 (9.19)	0.47
Ictus to randomisation, Median (IQR)	1 (1)	1 (2)	1 (1)	0.99 ¹
NIHSS (stroke patients only), Median (IQR)	2 (4)	4 (5)	2 (3)	0.03 ¹
SBP	154.8 (27.1)	156.9 (28.4)	152.7 (25.9)	0.44
DBP	81.5 (14.5)	83.8 (15.5)	79.2 (13.1)	0.11
HR	77.55 (17.5)	78.1 (18.0)	77.0 (17.1)	0.75
Alcohol units / week, Median (IQR)	3 (15)	4 (19)	2 (14)	0.24 ¹
Male sex	56 %	58.8 %	51.0 %	0.32
Qualifying event Stroke	68 %	68.6 %	67.3 %	0.89
Qualifying event TIA	32 %	31.4 %	32.7 %	0.89
TACS	14 %	19.6 %	8.2 %	0.15 ²
PACS	41 %	37.3 %	44.9 %	0.43
LACS	33 %	33.3 %	32.7 %	0.94
POCS	12 %	9.8 %	14.3 %	0.49
IHD	16 %	13.7 %	18.4 %	0.53
Hypertension	58 %	54.9 %	61.2 %	0.52
Diabetes	15 %	13.7 %	16.3 %	0.72
Smoker	32 %	35.3 %	28.6 %	0.47
Aspirin	55 %	62.7 %	46.9 %	0.11
Dipyridamole MR	21 %	27.5 %	14.3 %	0.10
Clopidogrel	27 %	21.6 %	32.7 %	0.21
Any antiplatelet	75 %	78.4 %	71.4 %	0.42

ACE-I	37 %	27.5 %	46.9 %	0.04
ARB	8 %	9.8 %	6.1 %	0.71 ²
Thiazide	18 %	15.7 %	20.4 %	0.54
CCB	15 %	7.8 %	22.4 %	0.05 ²
Beta-blocker	17 %	19.6 %	14.3 %	0.48
Alpha-blocker	2 %	2.0 %	2.0 %	1.00 ²
Statin	58 %	51.0 %	65.3 %	0.14
Other lipid modifying	2 %	2.0 %	2.0 %	1.00 ²
Hypoglycaemic therapy	10 %	9.8%	10.2 %	0.95
“Any duration” PAF detected (14 days)	8.0 %	7.8 %	8.2 %	1.00 ²
“Any duration” PAF detected (90 days)	16 %	11.8 %	20.4 %	0.24
“Sustained” PAF detected (14 days)	5 %	3.9 %	6.1 %	0.68 ²
“Sustained” PAF detected (90 days)	12 %	7.8 %	16.3 %	0.23 ²

Continuous baseline characteristic values are quoted as mean (SD) unless otherwise stated. Comparison was with two-sample t-test except when a variable did not approximate to the Normal distribution, when comparison with the Mann-Whitney test ¹ was used. Categorical baseline characteristics are quoted as % (95% CI). Comparison between groups (no interval versus interval), is with difference in 2 proportions. When observed frequencies were low, Fisher’s exact test ² was used. A p value of < 0.05 was considered statistically significant.

SP, standard practice; SP-AM, standard practice plus additional monitoring; CI, confidence interval; NIHSS, National Institute of Health Stroke Scale; IQR, inter-quartile range; SBP, systolic blood pressure, DBP, diastolic blood pressure; HR, heart rate; TACS, total anterior circulation syndrome; PACS, partial anterior circulation syndrome, LACS = lacunar anterior circulation syndrome; POCS, posterior circulation syndrome; TIA, transient ischaemic attack; IHD, ischaemic heart disease; MR, modified release; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; PAF, paroxysmal atrial fibrillation

Paroxysms of AF of “sustained” duration were observed in 14 of 49 individuals with interval R-testing (7 patients in each of the SP and SP-AM groups). Of these 14 cases, 7 patients (5 in the SP group, 2 in the SP-AM group) had not had sustained paroxysms identified during the 90 day follow up period during Study 1.

Paroxysms of AF of “any duration” were observed in 30 of 49 individuals with interval R-testing (16 patients in the SP group and 14 patients in the SP-AM group). Of these 30 cases, 16 patients (13 from the SP group, 3 from the SP-AM group) had not had AF identified during the 90 follow up period during Study 1.

Tables 3-7, 3-8 & 3-9 detail sensitivity, specificity, PPV & NPV of “sustained” and “any duration” PAF episodes detected by, respectively: initial R-test in the SP-AM group; SP investigations at 14 days (in each of the SP, SP-AM and combined groups); and any Study 1 investigation at 14 days (in each of the SP-AM and combined groups) for corresponding “sustained” and “any duration” PAF episodes detected on interval R-test. The predictive values of SP investigation and any Study 1 investigation, performed up to 90 days, are detailed in Appendix A; tables A1 & A2, respectively.

Study 1 R-test detection of PAF, performed in the 7 days following recruitment, carried high specificity and PPV for PAF detection on interval R-testing, for both “sustained” and “any duration” episodes. Sensitivity was however modest for subsequent interval detected episodes. NPV of initial R-test investigation was modest.

AF detection through Study 1 standard practice investigations also held high specificity for detection of AF on interval testing, particularly for episodes of “any duration”. Accordingly PPV of AF detected on SP investigations in study 1 was good. However, Study 1 SP investigations carried low sensitivity and poor NPV for subsequent interval R-test detectable PAF episodes, at both 14 and 90 day follow up points.

When AF was detected with any Study 1 investigation modality, specificity and PPV for subsequently identified AF on interval R-test was high. However, sensitivity was low, reflecting the low sensitivity of SP investigation. NPV value for subsequent interval detected episodes was also low.

Table 3-7: Sensitivity, Specificity, PPV, and NPV for initial R-test investigation detected AF, for presence of corresponding duration AF episodes after 90 days, in the SP-AM study group

	Sensitivity	Specificity	PPV	NPV
R-test “Sustained”	57 (18 - 90)	100 (83 - 100)	100 (47 - 100)	84 (60 - 97)
R-test “any duration”	64 (35 - 87)	100 (72 - 100)	100 (72 - 100)	64 (35 - 87)
Data are presented as % (95% CI). PPV, positive predictive value; NPV, negative predictive value				

Table 3-8: Sensitivity, Specificity, PPV, and NPV for SP investigation detected AF at 14 days, for presence of corresponding duration AF episodes after 90 days, in the SP, SP-AM and combined study groups

	Sensitivity	Specificity	PPV	NPV
SP group 14 “Sustained” PAF	14 (0 - 58)	100 (85 - 100)	100 (5 - 100)	76 (55 - 91)
SP group 14 “any duration” PAF	6 (0 - 32)	100 (74 - 100)	100 (5 - 100)	40 (21 - 61)
SP-AM group 14 “Sustained” PAF	29 (4 - 71)	100 (83 - 100)	100 (22 - 100)	76 (52 - 92)
SP-AM group 14 “any duration” PAF	21 (5 - 51)	100 (72 - 100)	100 (37 - 100)	45 (23 - 69)
Combined group 14 “Sustained” PAF	21 (5 - 51)	100 (92 - 100)	100 (37 - 100)	76 (61 - 87)
Combined group 14 “any duration” PAF	13 (4 - 31)	100 (85 - 100)	100 (47 - 100)	42 (28 - 58)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table 3-9: Sensitivity, Specificity, PPV, NPV for all combined Study 1 investigation detected AF at 14 days, for presence of corresponding duration AF episodes after 90 days, in the SP-AM and combined study groups

	Sensitivity	Specificity	PPV	NPV
SP-AM Group 14 “Sustained” PAF	57 (18 - 90)	100 (83 - 100)	100 (47 – 100)	84 (60 - 97)
SP-AM Group 14 “any duration” PAF	64 (35 - 87)	100 (72 - 100)	100 (72 - 100)	64 (35 - 87)
Combined Group 14 “Sustained” PAF	36 (13 – 65)	100 (92 – 100)	100 (55 – 100)	80 (65 – 90)
Combined Group 14 “any duration” PAF	37 (20 – 56)	100 (85 – 100)	100 (76 – 100)	50 (33 – 67)
Data are presented as % (95% CI). SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Tables 3-10, 3-11 & 3-12 detail sensitivity, specificity, PPV & NPV of “any duration” PAF episodes detected by, respectively: initial R-test in the SP-AM group; SP investigations at 14 days (in each of the SP, SP-AM and combined groups); and any Study 1 investigation at 14 days (in each of the SP-AM and combined groups), for “sustained” PAF episodes detected on 90-day interval R-test. The predictive values of SP investigation and any Study 1 investigation, performed up to 90 days, are detailed in Appendix A; tables A-3 and A-4, respectively.

Study 1 R-test detection of “any duration” PAF, performed in the 7 days following recruitment, carried moderate specificity and PPV for “sustained” PAF detection on interval R-testing. Sensitivity was moderate and NPV good for interval R-test detected “sustained” PAF episodes.

“Any duration” AF episode detection through Study 1 standard practice investigations held good specificity and moderate PPV for detection of “sustained” PAF episodes on interval testing. Sensitivity was poor however, with moderate NPV. When “any duration” PAF episodes were detected with any Study 1 investigation modality, specificity, sensitivity, PPV and NPV were all moderate for subsequent interval detected “sustained” PAF episodes.

Table 3-10: Sensitivity, Specificity, PPV, NPV of “any duration” PAF detection on initial R-test investigation in the SP-AM study group, for “sustained” duration PAF on interval R-test investigation after 90 days

	Sensitivity	Specificity	PPV	NPV
SP-AM initial R	86 (42 – 100)	81 (54 – 96)	67 (30 – 93)	93 (66 – 100)
Data are presented as % (95% CI). SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table 3-11: Sensitivity, Specificity, PPV, NPV of “any duration” PAF detection on SP investigation at 14 days, in the SP, SP-AM and combined study groups, for “sustained” duration PAF on interval R-test investigation after 90 days

	Sensitivity	Specificity	PPV	NPV
SP-AM Group SP 14	29 (4 – 71)	94 (70 – 100)	67 (9 – 99)	75 (51 – 91)
SP Group SP 14	14 (0 – 58)	100 (85 – 100)	100 (5 – 100)	76 (55 – 91)
Combined Group SP 14	21 (5 – 51)	97 (85 – 100)	75 (19 – 99)	76 (61 – 87)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table 3-12: Sensitivity, Specificity, PPV, NPV of “any duration” PAF detection on all combined Study 1 investigations at 14 days, in the SP-AM and combined study groups, for “sustained” duration PAF on interval R-test investigation after 90 days

	Sensitivity	Specificity	PPV	NPV
SP-AM Group initial R & SP 14	86 (42 – 100)	75 (48 – 93)	60 (26 – 88)	92 (64 – 100)
Combined Group R & SP 14	50 (23 – 77)	89 (73 – 97)	64 (31 – 89)	82 (66 – 92)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

3.2.4. Discussion

This sub study examined the predictive value of testing for occult AF in the immediate aftermath and up to 90 days after acute ischaemic stroke or TIA, relative to AF detection by non-invasive cardiac event monitoring evaluation performed 90 days following the index event.

The study had limitations including sample size and potential for bias due to convenience sampling. Nevertheless, the results suggest that following ischaemic stroke & TIA, early detection of AF, including within the initial 7 days of ictus by R-test event monitoring and within 14 days by SP investigations, holds high PPV for detection the of AF by R-test monitoring beyond 3 months of event ictus. These findings are in keeping with the observation that AF, detected at the time of clinical presentation with ischaemic stroke by admission 12-lead ECG, is associated with subsequently detectable AF ²⁸⁸ and provide support for basing treatment decisions on the detection of AF in the period immediately after stroke. In addition, the results indicate that the NPV of a single episode of non-invasive event monitoring may be insufficient to justify performing any additional investigation for AF.

It has been suggested that the occurrence of AF early following stroke may be attributable to a stress response to either the neurological insult or an associated medical complication (e.g. sepsis, acute coronary syndrome (ACS)). Alternatively cortical ischaemia may precipitate the dysrhythmia ^{124, 285, 286}. As these stressors will resolve in survivors over time, such observation of AF may be a transient phenomenon only, with low likelihood of persistence or recurrence on follow up. Thus, demonstrable AF may neither reflect the aetiological mechanism for the clinical presentation nor confer elevated risk of cardioembolic recurrent events ²⁸⁷. In such cases, institution of anticoagulant therapy would not be expected to confer additional benefit to a patient with restored and maintained sinus rhythm ²⁵².

Whilst having a conceptual basis, such concerns are relatively poorly evidenced. Secondary prevention studies which have demonstrated benefit of anticoagulant therapy in patients with AF did so having included patients for which evidence of AF was predominantly based on the admission ECG at the point of presentation with stroke or TIA ^{135, 137}. Only a minority of patients were included on the basis of antecedent history of AF. No interval ECGs were

required to confirm AF in the EAFT which demonstrated benefit of anticoagulation on this basis. Nevertheless, it is reassuring to confirm that demonstrable AF in the period immediately following ischaemic stroke & TIA, detected by prolonged monitoring, corresponds to persistent evidence of PAF after a 90 day interval.

The findings of this sub study reinforce those of the first RCT described in Chapter 3.1: standard practice investigative procedures based on existing guidelines appear inadequate in detecting occult PAF. The sensitivity of SP investigation to interval R-test detectable AF was poor in each of the SP, SP-AM and combined study population groups. Consequently, the NPV of SP investigation for interval detected AF was also poor.

Detection of AF, by either SP investigation or through the initial R-test, was highly specific for AF detected on interval R-testing, with a high PPV. The sample size was modest such that confidence intervals were wide. However, all patients in whom initial event monitoring detected AF also had demonstrable AF on interval monitoring, for both paroxysms of “sustained” and “any duration”. Thus, with greater sensitivity than SP investigative procedures and high specificity, the sub study results re-affirm the findings in Study 1, supporting use of cardiac event monitoring for the detection of PAF following ischaemic stroke & TIA over existing guideline approaches.

This finding provides reassurance that the observation of higher AF detection in the SP-AM group in Study 1 did not reflect a chance observation attributable to higher AF prevalence in that group: the proportion of patients in the SP group with interval demonstrable AF was similar to that in the SP-AM group in Study 1 and on interval R-test follow up.

It is noteworthy that the NPV of the initial R-test was good but not high enough that clinicians would necessarily be assured that no further investigation for AF was required. This study lacked power to examine which factors might indicate the necessity for repeated monitoring. Invasive cardiac event monitoring has been proposed as a means to investigate patients for occult PAF in cryptogenic stroke ²⁸⁶. The findings of additional diagnostic yield with interval R-test monitoring in this study might indicate that simpler strategies, including repeated or prolonged monitoring, could be effective in this regard.

Given that the significance of briefer paroxysms of AF in conferring risk remains uncertain ^{252, 256}, this study explored the predictive value of detection of “any duration” AF episodes, through initial Study 1 investigations, for “sustained” episodes on subsequent interval cardiac event monitoring. The NPV of this approach was high for initial R-test investigation. I.e. if the initial R-test failed to identify episodes of “any duration” PAF, it was unlikely that more “sustained” episodes would be evident on interval testing. However, the PPV was only modest, such that “any duration” episodes on initial testing were only associated with “sustained” duration episodes on interval testing in two thirds of patients. SP investigation detection of “any duration” PAF were highly specific for subsequent interval detected “sustained” episodes. However, NPV was low, reflecting the overall poor sensitivity of the SP investigation approach.

It is worth considering that the natural history in AF is progression from PAF to the more sustained subtypes of AF over time ^{244, 297}. The studies to have differentiated risk between subtypes had relatively short follow-up ²⁴⁸. Assuming patients undergo transition between subtypes, it is reasonable to assume that more prolonged follow up would minimise any difference observed between subtypes at baseline.

Limitations

The main limitation of this study is that the available sample represented a convenience sample from a larger study population. However, all patients were invited to participate and approximately 50% of the eligible population was included. Those included in the interval analysis did not appear to differ significantly from those who were not, in either baseline characteristics or the frequency with which AF was detected in the period immediately following ischaemic stroke or TIA. The notable exception to this was that the NIHSS amongst stroke patients was lower amongst those in the interval analysis, potentially reflecting greater ease in repeated hospital attendance amongst those with milder stroke severity. ACE-I and CCB treatment were each higher in the interval R-test group at baseline. This difference would not immediately raise suspicion of intrinsic differences in observation of AF at interval follow up.

Secondly, detection of both “sustained” or “any duration” paroxysms of AF, through standard practice investigation modalities, did not differ between those with and without R-test interval testing. Nevertheless, there is potential for bias to have arisen in this regard which might potentially limit the generalisability of results. By chance, patients who attended for interval R-test may have had a greater propensity to exhibit AF on subsequent testing (due to intrinsic propensity, particular medication use or development of other predisposing risk factors for AF).

A third limitation is the sample size available for the interval R-test analysis. Though point estimates suggested clinically relevant information is yielded from initial investigation in terms of PPV, confidence intervals were wide. A larger sample size would provide greater precision and also permit exploration of subgroups and potential identification of factors associated with reduced specificity of early investigation.

Finally, the clinical relevance of assessing predictive value of early detectable AF compared with interval detected AF relies on the assumption that R-test detected AF distant from the index event holds clinical relevance. As discussed in chapter 2.5 and 2.6, there is no convincing evidence which undermines the available clinical trial data supporting use of anticoagulant therapy in patients with “sustained” duration paroxysms of AF detected in the aftermath of acute stroke or on interval testing.

Conclusions

This study provides evidence that AF identified in the period immediately following stroke is likely to be evident on interval testing, performed 3 months after ictus. This provides a supportive basis for early investigation and treatment decisions based on early identification of cases. Despite the small sample size, the NPV of SP investigation is poor and is only modest for early R-test monitoring. Together, these findings support the conduct of early cardiac event monitoring investigation in all patients and point toward a potential need for repeated interval monitoring.

Chapter 4

The potential role of uric acid and xanthine oxidase in the pathogenesis and pathophysiology of cardiovascular disease

An association between elevated serum uric acid (SUA) levels and increased risk of cardiovascular events & mortality has been described in the literature for several decades²⁹⁸. This has led many authors to hypothesise the existence of an aetiological relationship between UA and atherosclerotic disease. In recent years, a second hypothesis has been proposed to explain the relationship between elevated SUA and increased risk of cardiovascular disease; Rather than UA holding a direct atherogenic role per se, SUA has been proposed as representing a surrogate marker for harmful levels of vascular oxidative stress, produced by the xanthine oxidase (XO) enzymatic system²⁹⁹.

However, such an aetiological relationship remains unproven and a source of debate. There is inconsistency in the strength of the association reported between epidemiological studies. In addition, SUA elevation clusters with traditional CV risk factors, leading some commentators to cast doubt on the validity of the epidemiological associations representing a novel aetiological relationship. Moreover, definitive studies evaluating these hypotheses have not been forthcoming. Consequently, the potential therapeutic strategy of UA reduction through XO inhibition (XOI) in the prevention of cardiovascular disease remains an area that awaits definite evaluation.

4.1. Biochemistry of uric acid and the xanthine oxidase enzymatic system

DNA and RNA molecules undergo degradation to purine nucleotides and bases. Adenine and Guanine are subsequently either recycled in nucleic acid synthesis processes or undergo further metabolism prior to excretion. Uric acid represents the final metabolic product in the degradation pathway of these purines³⁰⁰.

Adenine is formed from IMP (which itself is derived from AMP). Removal of a ribose unit from Adenine results in the formation of Hypoxanthine. Guanine is formed from GMP and subsequently undergoes deamination, resulting in the formation of xanthine. Subsequently, under the activity of the enzyme XO, hypoxanthine is oxidized, resulting in the formation of xanthine. Xanthine, produced by either pathway, is further oxidized to UA, again under the activity of the enzyme XO. In most non-human mammals, under the action of the enzyme

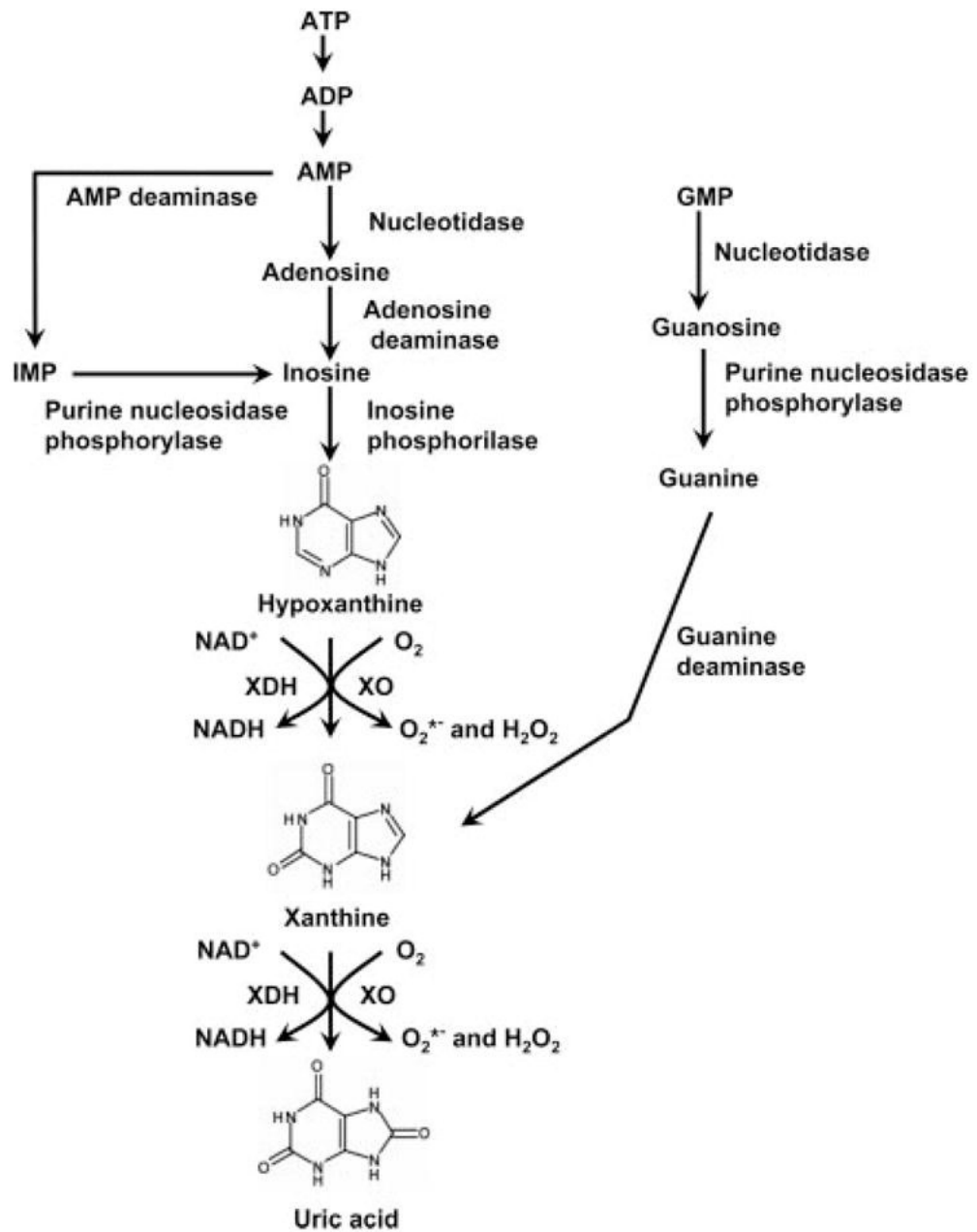
uricase, UA undergoes further metabolism to Allantoin, which is then excreted in urine. However, humans and higher primates lack uricase and UA undergoes no further metabolism. It is excreted via predominantly renal elimination.

Xanthine oxidase is from the molybdenum iron-sulfur flavin hydroxylase group of enzymes and is found predominantly in the liver and gastrointestinal tract but also in the kidney and brain. It is also found throughout the cardiovascular system³⁰¹. While the major role of XO is conversion of hypoxanthine and xanthine to UA, an interconvertible form, xanthine dehydrogenase, also exists and is responsible for conversion of NAD⁺ to NADH³⁰². XO activity is associated with production of reactive oxygen species (ROS), including hydrogen peroxide and superoxide. ROS add to and may initiate, through reaction with NO and formation of peroxynitrite, vascular oxidative stress³⁰³.

Sources of purine substrate for the XO metabolic pathway include endogenous DNA and RNA, and exogenous ingested dietary purines (e.g. in alcoholic beverages and red meat). Thus, SUA may be increased through conditions in which cellular turnover is increased, with high levels of DNA and RNA degradation (e.g. tumour lysis syndromes) or in the context of increased dietary exposure³⁰⁴.

Serum UA levels are determined by excretion through the kidneys. Excretion may also occur through the intestinal tract but does not appear subject to regulation or variation. Uric acid is filtered at the renal glomerulus but may be reabsorbed in the proximal convoluted tubule under the action of the URAT1 transporter³⁰⁵. Inter individual variations in SUA in the setting of stable dietary intake are likely to be attributable to variable renal excretion^{305, 306}. SUA is typically higher in males than females and rises in females with ageing. Discrepancies between the sexes and following the menopause suggest a responsiveness of SUA to sex hormones^{307, 308}.

Figure 4-1: Illustration of purine metabolism
(reproduced from Pacher et al, Pharmacol Review 2006)



Abnormal elevations in UA are associated with a number of medical morbidities, particularly conditions which predispose to the development of cardiovascular events, including renal impairment³⁰⁰. SUA is typically elevated in the context of DM³⁰⁹ and increased body mass index (BMI)³¹⁰. These observations, together with findings of HU in states of relative insulin resistance^{311, 312}, have led to a hypothesis that UA levels may either reflect, or conversely mediate, insulin sensitivity. SUA is also found to be elevated in the context of hypertension^{313, 314} dyslipidaemia³¹⁵, smoking³¹⁶ and is a component of the metabolic syndrome³¹⁷. Levels are consistently elevated in both renal³¹⁸ and cardiac dysfunction. SUA is also elevated amongst patients treated with diuretics³¹⁹.

4.2. Association of serum uric acid with incident cardiovascular disease

Epidemiological studies have reported an association between UA levels and risk of cardiovascular disease and mortality for several decades. Over this period, the majority of published studies have reported a positive association, though not universally so. Conflicting evidence has been attributed to differences in study methodology, particularly adjustment for risk factors and medical therapy and to differences in the populations studied. More recently, attempts have been made to combine the available evidence in meta-analysis.

The largest single prospective cohort study published to date, included nearly 90,000 healthy Taiwanese individuals aged over 35 years. During mean follow-up of 8 years, 5,427 deaths occurred. Those with hyperuricaemia (defined as SUA >7mg/dl), were at increased risk of all-cause mortality, combined cardiovascular disease events and stroke. This association remained significant following adjustment for conventional CV risk factors and was also observed within subgroups with specific CVRFs, including hypertension and diabetes³²⁰. The NHANES cross sectional population study included 5926 healthy North American individuals, aged 25 - 74 years, with available SUA measurements. During mean follow up of 16.4 years, IHD deaths were increased amongst those individuals in the highest compared with the lowest UA quartile at baseline (risk ratio 1.77 95% CI 1.08 - 3.98)³²¹. Stack and colleagues reported an analysis based on the NHNESIII study population, which included 15,773

participants³²². For every 1mg/dl increase in SUA, both ACM and CVM were significantly increased.

SUA has also been reported to predict both mortality and cardiovascular events in populations with either established CV disease or risk factor conditions for cardiovascular events. Amongst the subgroup of individuals in the NHNESIII study with DM, elevated SUA was associated with ACM³²³. SUA has been found to predict ACM amongst patients with CAD^{324, 325}, and to predict CVM in patients with hypertension³²⁶. SUA is predictive of cardiovascular events in those with hypertension³²⁷⁻³²⁹, and stroke and diabetes^{330, 331}.

Several studies have reported the relationship between SUA and risk of stroke as a specific CV outcome. Chen & colleagues observed increased ischaemic stroke related mortality in Healthy Taiwanese individuals³²⁰ and Strasak and colleagues reported similar findings in an Australian population³³². A positive association with stroke related mortality has been observed in hypertensive populations³²⁶. In patients with prior stroke, risk of recurrent stroke events, are associated with increased SUA³³³ as is the risk of recurrent combined vascular events³³⁰, including in the subgroup of patients with DM³³¹.

However, not all published studies have reported an independent association between SUA and subsequent risk. Culleton and colleagues, performed an analysis of 6763 individuals in the Framingham population study³³⁴. During 23 years follow up, 1460 deaths occurred of which 429 were characterised as being of CV aetiology. Unadjusted analysis suggested a highly significant association of increasing SUA with risk of both ACM and CVM. However, following adjustment for age, sex, traditional CVRFs, including measures of obesity and insulin resistance and diuretic based therapy, no significant association was observed. In males, following adjustment, the direction of correlation was reversed toward a negative association of risk with SUA. The authors concluded that UA merely represents a surrogate marker for risk attributable to established CV risk factors and use of therapies, which may in turn indicate risk factor disease severity. In a Korean population of 22,698 individuals aged 30-77 years, Jee and colleagues also observed a non-significant association between SUA and subsequent ACM and CVM³³⁵.

Explanations for the conflicting findings reported between population studies are not readily forthcoming. Similar methodology to that employed in the Framingham analysis, including adjustment for the same co-morbidities and therapeutic burden, has subsequently resulted in identification of positive associations in other populations^{320, 321, 332, 336}.

SUA has been found to hold independent predictive value within populations with specific cardiovascular risk conditions which were adjusted for in the general population analyses^{321, 336}. This could potentially indicate SUA truly holding independent predictive value. An alternative explanation includes the possibility that SUA represents a surrogate marker for severity of derangement for each of these established risk factors.

Of note, several studies have identified sex specific differences in the relationship between SUA and risk of mortality and of CV events. The relationship appears to be attenuated in males relative to females^{320, 321}. A meta-analysis, including data from 26 studies and 402,997 adults, reported that HU was associated with both CHD incidence and mortality in the population as a whole³³⁷. However, no significant association was associated in the male population. In addition, the relationship between SUA and risk in females appears to be stronger in post-menopausal individuals³³⁸. These findings may suggest an interaction between sex hormones and the influence of UA.

In some studies, the relationship between SUA and risk appears to be J-shaped, with some recrudescence of risk at the lowest levels of SUA³³⁹⁻³⁴². Theoretical explanations include reference to physiological levels of UA potentially holding beneficial effects. This possibility is discussed in greater detail below.

Despite failure to identify an independent relationship between SUA and outcomes in selected populations, the balance of published evidence appears supportive of such a relationship. In a meta-analysis of prospective study data, including 172,123 participants, Zhao and colleagues found that elevated SUA was associated with both increased ACM and CVM³⁴³. There was limited data available for very low levels of SUA from which to derive strong conclusions.

A systematic review and meta-analysis of prospective cohort studies which included risk of stroke incidence in relation to SUA level was reported by Kim and colleagues in 2009³⁴⁴. The

authors identified 16 such studies, totalling 238,499 individuals. Hyperuricaemia was associated with both increased stroke incidence (6 studies; RR 1.41, 95% CI 1.05, 1.76) and mortality (6 studies; RR 1.36, 95% CI 1.03, 1.69) (unadjusted analyses). An analysis based only on studies which included adjustment for conventional CV risk factors, also indicated increased risk with hyperuricaemia for both stroke incidence (4 studies; RR 1.47, 95% CI 1.19, 1.76) and mortality (6 studies; RR 1.26, 95% CI 1.12, 1.39).

Table 4-1 summarises the reported associations between elevated SUA and risk of stroke. Table 4-2 summarises the reported associations between elevated SUA and risk of all-cause mortality, cardiovascular mortality and cardiovascular events in patients with established CV disease, CV risk factors and general populations. Risk associations have typically been reported for incremental increases in SUA concentration or alternatively as comparative risk between patients with highest stratification of SUA level to those with the lowest SUA level (e.g. tertiles, quartiles, quintiles).

Table 4-1: Relationship between SUA and risk of stroke

Study	Population	Risk of Stroke Outcome
Lehto	Diabetes	HR 1.91 (1.24-2.94) for stroke ^a
Wang	Hypertension	HR 1.34 (1.14-1.57) for fatal stroke ^b
Chen	Healthy Volunteers	HR 1.35 (1.04–1.76) for IS related mortality ^c HR 1.09 (1.05–1.14) for IS related mortality ^d
Strasak	Healthy Volunteers	HR 1.37 (1.09-1.74) for stroke related mortality ^a HR 1.07 (1.01–1.13) for stroke related mortality ^e
Schretlen	Healthy Volunteers	OR 2.6 (1.2-5.4) for white matter hyperintense signals on MRI imaging ^a
Results expressed as ratio and 95% CI. a = for highest vs. lowest group; b = for each 50µmol/L increment in SUA; c = SUA > 7 mg/dl vs. < 7 mg/dl; d = per additional 0.1mmol/l in SUA; e = for each 59.48 micro mol increase in UA level. HR, hazard ratio; OR, odds ratio; IS, ischaemic stroke; MRI, magnetic resonance imaging		

Table 4-2: Relationship between SUA and cardiovascular risk

Reference	Population	Risk of CV Outcome
Weir	Acute stroke	RR 1.27 (1.18-1.36) for recurrent vascular events ^a
Newman	Diabetes & stroke	HR 1.49 (1.21-1.84) for recurrent CV event ^a
Madsen	Coronary Disease	HR 1.5 (1.02-2.1) for all-cause mortality ^b
Bichel	Coronary Disease	HR 1.23 (1.11-1.36) for all-cause mortality ^c
Franse	Hypertension	HR 1.32 (1.03-1.69), for CV events ^b
Alderman	Hypertension	HR 1.22 (1.11-1.35) for CV disease ^d
Wang	Hypertension	HR 1.14 (1.02-1.27) for CV mortality ^e
Verdaccia	Hypertension	HR 1.73 (1.01-3) for CV event rates ^b
de Leeuw	Hypertension	HR 1.03 (0.93-1.14) for CV mortality ^e HR 1.06 (0.99-1.13) for all CV events ^e
Fang	Healthy individuals	HR 1.109 (1.02 - 1.18) for CV mortality ^f
Chen	Healthy individuals	HR 1.16 (p<0.001) for all-cause mortality ^b
Strasak	Healthy individuals	HR 1.35 (1.20-1.52) for CV mortality ^b HR 1.37 (1.09-1.74) for stroke ^b
Meisinger	Healthy individuals	HR 1.40 (1.13-1.74) ACM ^a
Culleton	Healthy individuals	HR 0.98 (0.78 – 1.24) ^a & 0.97 (0.91-1.03) ^{f,*} for ACM HR 1.16 (0.89 – 1.51) ^a & 1.03 (0.97-1.09) ^{f,#} for ACM
Stack	General population	HR 1.16 (1.10-1.22) ^f (ACM) & 1.16 (1.10-1.22) (CVM) ^f
Results expressed as ratio and 95% CI. a = per additional 0.1mmol/l in serum UA, b = for highest vs. lowest group, c,d = per additional 0.6 and 0.86mmol/l in SUA respectively, e = for each 50µmol/L increment in SUA. f = for each 59.48 micro mol increase in UA level. * = male. # = female. RR, relative risk; CV, cardiovascular; HR, hazard ratio; AM, all-cause mortality; CVM, cardiovascular mortality.		

4.3. Putative vasculopathic role for uric acid

Uric acid crystals have the potential to induce profound inflammation, evident in their role in the pathogenesis of gout. UA holds properties which may contribute to the pathogenesis of atherothrombotic disease exist at each of the molecular, cellular and tissue levels and is associated with several surrogate markers of impaired cardiovascular health and function.

Uric acid is associated with increased oxygenation of LDL³⁴⁵, a key step in atherogenesis. HU is associated with induction of vasoconstrictive mediators including endothelin-1³⁴⁶ and angiotensin II³⁴⁷. Uric acid crystals have also have been shown to stimulate release of the platelet constituents serotonin, ATP and ADP³⁴⁸. In addition, UA has been associated with elevated levels of free radical formation³⁴⁹ and elevated levels of oxidative stress^{350, 351}. As discussed below, these latter findings may reflect not only direct effects of uric acid per se, but also the activity of the XO enzymatic system. UA has been implicated in reduced insulin sensitivity³⁵². Serum UA levels have also been linked to levels of pro-inflammatory cytokines and may have a role in perpetuating the inflammatory response that characterises atherosclerosis.

At the cellular level, HU is associated with promotion of vascular smooth muscle cell proliferation³⁵³. Platelet function is altered by UA, with evidence of increased adhesiveness³⁴⁸. Hyperuricaemia is also associated with endothelial dysfunction⁷⁴.

Uric acid has a putative role in the development of hypertension, via effects on nitric oxide (NO) production in the macula densa^{354, 355}, renal salt handling³⁵⁶ and effects on the Renin Angiotensin system³⁵⁷. Aside from the association with cardiovascular events and mortality in healthy individuals, UA also appears to be independently predictive of new onset hypertension³⁵⁸. Studies have shown that UA reduction with allopurinol can improve BP in adolescents with newly diagnosed hypertension³⁵⁹.

In the Korean Multi-Rural Communities Cohort study, the relationship between SUA and surrogate markers of CV health was examined in 5,568 individuals. Ba-PWV was increased amongst individual with hyperuricaemia, with a positive correlation and linear relationship observed in both male and female individuals. Serum UA has also been related to markers of

arterial stiffness in other populations, including post-menopausal women ³⁶⁰, Japanese individuals ³⁶¹ and patients with cerebrovascular disease ³⁶². Conversely, other studies have failed to identify an association between SUA and arterial stiffness ^{363, 364}.

In the ARIC study, SUA was related to CIMT but only in male individuals ³⁶⁵. Tavit and colleagues found that in a population of hypertensive patients, CIMT was increased amongst patients with HU and that SUA was independently associated with CIMT ³⁶⁶. Conversely, in the Korean Communities cohort study, no relationship between SUA and CIMT was observed ³⁶⁷. In a population of 8144 Japanese individuals undergoing health screening, SUA was independently associated with increased odds of presence of carotid plaques ³⁶⁸.

4.4. Uric acid - a surrogate marker for vascular oxidative stress

The observation of clustering of HU with established CVRFs has led to speculation that UA may represent a surrogate marker for increased risk of cardiovascular disease which is attributable to the presence of other established risk factors. Authors have proposed that UA potentially represents an embedded component of traditional risk factors ³⁶⁹ and as such may not itself carry any individual mediating role *per se*. Conversely, as discussed above, UA holds atherogenic properties which might explain the observed epidemiological associations. An alternative explanation might be that UA represents a surrogate marker for an alternative, novel mediator of increased risk: increased levels of harmful vascular oxidative stress produced by activity of the XO enzymatic system.

The role of ROS in the pathogenesis of cardiovascular disease has been extensively described ³⁷⁰⁻³⁷³. Through contributing to endothelial dysfunction, oxidative stress is considered an early event in pathogenesis of atherosclerotic disease. As a byproduct of purine metabolism to UA, activity of XO also results in generation of superoxide anions, and represents one of the principle sources of ROS in the human vasculature ^{374, 375}.

Traditionally, XO has been considered less important than NADPH oxidase as a source of oxidative stress in the vasculature. Emerging evidence suggests the reverse may be more representative of the *in vivo* situation ³⁷⁶. Studies suggest that XO activity is greatly increased

in those with heart failure and in response to ischaemic conditions^{377, 378}. In these studies, Landmesser and colleagues demonstrated that Angiotensin II increases endothelial expression of XO. Moreover, endothelial XO activity was reduced with an ARB (Losartan), which also ameliorated the beneficial effect of Oxypurinol on endothelial function, suggesting that the mechanism of ARB benefit may, in part, be related to reduced XO activity. Expression of an endothelial bound form of XO has been shown to increase in ischaemia and in response to increased levels of pro-inflammatory cytokines³⁷⁹. On a practical level, the potential clinical relevance of XO as a source of oxidative stress is much greater than other contributing pathways such as NADPH oxidase; a licensed and commonly used inhibitor of XO exists, with a defined tolerability and safety record.

The hypothesis that increased SUA may be a surrogate for increased CV risk conferred through XO mediated oxidative stress, is supported by clinical trials data in patients with CHF²⁹⁹. In this population, reduction of UA with the uricosuric agent Probenecid yielded no improvement in endothelial dysfunction. Conversely, similar magnitude of UA reduction achieved through XOI with Allopurinol, resulted in significant improvement in endothelial function. These findings suggest that the mechanism of UA reduction may be more relevant than uric acid reduction *per se*.

4.5. Uric acid and xanthine oxidase - physiological & pathophysiological properties

The hypothesis that elevated SUA levels are harmful to the vasculature conflicts with a key property of UA: it is an effective anti-oxidant, with activity against peroxynitrite and hypochlorous acid (but not superoxide, a by-product to its own production)^{380, 381}. During evolution, humans appear to have lost the ability to make the uricase enzyme and developed the ability to actively reabsorb UA in the kidney. Both these mechanisms contribute to the higher SUA levels seen in humans. The human life span has increased during evolution and this may in part reflect a reduction in early cancer rates compared to other mammalian species. It has been argued that this is due to evolution of more effective antioxidant mechanisms and that higher levels of SUA are a key component of such mechanisms³⁸², UA

being as effective as ascorbic acid in this regard. Given that UA is the most abundant antioxidant in plasma, it is feasible that this change in UA metabolism has been beneficial and contributed to our prolonged survival.

This hypothesis is further supported by data showing that in healthy human volunteers, UA administration increases total serum antioxidant capacity³⁸³ and reduces oxidative stress associated with exercise³⁸⁴. The systemic administration of UA has also been demonstrated to improve endothelial function³⁸⁵.

Under pathophysiological circumstances, the antioxidant properties of UA ought to confer potential benefit. In the context of acute ischaemia, antioxidant activity could be considered highly beneficial in reducing tissue damage. SUA levels increase after an ischaemic insult, including within the brain in animal models of cerebral ischaemia^{386, 387}. Many argue that elevated SUA represents a physiological and protective response to oxidative stress and acute vascular insults^{388, 389}. In a transient ischaemia model, infusion of UA led to a reduction of infarct volume and improved behavioural outcome³⁸⁷ suggesting therapeutic potential of SUA in conditions of acute ischaemia. Similar findings were demonstrated in models of traumatic brain injury and multiple sclerosis^{390, 391}.

This hypothesis is potentially supported by the observation in 800 patients with acute ischaemic stroke that increasing SUA levels were associated with a good outcome (OR 1.12, 95% CI 1 -1.25, per additional mg/dl SUA) at seven days³⁹². However, epidemiological evidence is conflicting. In another population with acute stroke, reduced likelihood of favourable outcome at 90 days was associated with elevated baseline SUA (OR 0.78, 95% CI 0.67-0.91 per additional 0.1 mmol/L SUA) and an increased risk of recurrent vascular events was also observed³³⁰. This association was more prominent in those with diabetes³³¹. Others have suggested increased risk of early clinical deterioration following ischaemic stroke³⁹³ in those with increased SUA level. In another acute stroke population, increasing UA levels did link with increased odds of poor outcome (but not in an independent fashion) and there was no evidence that increasing SUA conveyed protection to the ischaemic brain³⁹⁴ and others have recently shown a risk with increased death early death after stroke³⁹⁵. Table 4-3 summarises the studies which have examined the relationship between SUA and outcome following stroke.

Recently, the therapeutic potential of UA administration after stroke has been explored in humans. In a small study of individuals treated with intravenous thrombolytic therapy, intravenous infusion of UA in the early period after ischaemic stroke reduced markers of oxidative stress³⁹⁶.

Table 4-3: Relationship between serum uric acid in acute stroke and clinical outcome

Reference	Population	Change in Outcome Measure
Cherubini	Acute stroke	Early clinical deterioration with higher SUA levels (p=0.001)
Karagiannis	Acute Stroke	OR 1.37 (1.13-1.67) for early death ^a
Chamorro	Acute Stroke	OR 1.12 (1-1.25) for good clinical outcome ^b
Dawson	Acute Stroke	OR 1.57 (1.02-2.42) for poor clinical outcome ^c
Newman	DM and stroke	HR 1.49 (1.21-1.84) for recurrent CV event ^d
Results expressed as ratio and 95% CI. a = for highest vs. lowest group; b = for each 59.48 micro mol increase in UA level; c = on univariate analysis; d = per additional 0.1mmol/l in SUA. SUA, serum uric acid; OR, Odds ratio, HR, Hazard ratio; DM, diabetes mellitus; CV, Cardiovascular		

Thus, whilst a consistent observation of increased risk of cardiovascular events has been observed for elevated SUA in a variety of populations, the influence of SUA in the context of an acute ischaemic event is less certain and brings in to focus the issue of the potential role of both UA and XO in both physiological and pathophysiological conditions.

The epidemiological evidence does suggest that elevated SUA links with increased incidence and severity of a variety of cardiovascular diseases. Data exist to support detrimental and prothrombotic effects of UA on platelet and endothelial function and a growing number of clinical studies suggest that UA lowering strategies improve surrogate markers of vascular risk, although for the most part the evidence concerns use of allopurinol which may have

other beneficial effects; XO mediated oxidative stress may play a significant role in the development of atherosclerosis. Yet we also know that UA has antioxidant properties and small pre-clinical and clinical studies suggest that SUA may be neuroprotective. Thus, on initial inspection, UA appears to hold both vasculoprotective and vasculopathic properties. Its enzymatic source of production may also be vasculopathic.

It is important to note that these hypotheses are not mutually exclusive. Firstly, increased local tissue levels of UA in ischaemia and brain injury may reflect levels of oxidative stress and XO activity, and thus the mechanism of harm and not an innate protective response. Essentially, the substance itself may well have antioxidant properties but its generation and associated superoxide anion production may be of much greater significance and detriment in both acute ischaemia (during which XO activity is increased and is likely to contribute to ischaemic and reperfusion injury)³⁹⁷ and the longer term. Whilst the antioxidant properties of UA could well be harnessed to improve clinical outcomes in the acute phase of cardiovascular and neurological illness, inhibition of the enzymatic system responsible for endogenous UA production might also be beneficial.

Furthermore, the measurement of UA levels in those at risk of disease may identify those at high risk who may benefit from treatments such as allopurinol, either because of UA itself or XO activity and oxidative stress. Data from the OPT-CHF study supports the concept of a need for targeted reduction of UA and / or XO; benefit from UA reduction may only be seen in those with SUA high enough to harm platelet and endothelial function while UA reduction in those with lower levels may compromise plasma oxidant activity such that this could be of detriment³⁹⁸.

4.6. Therapeutic strategies: Uric acid reduction and xanthine oxidase inhibition

There are several drugs which reduce SUA through varying mechanisms. Uricosuric agents reduce SUA through increased renal excretion (e.g. Probenecid, Sulfipyrazone). Fenofibrate³⁹⁹ and Losartan⁴⁰⁰ also possess modest uricosuric activity. Breakdown of UA to

Allantoin, as occurs in non-primate mammals, is feasible with exogenous recombinant urate-oxidase enzyme (rasburicase), resulting in reduction of SUA. Reduction in SUA may also be achieved through reduced production via inhibition of the XO enzymatic system (e.g. with Allopurinol, Oxypurinol or Febuxostat).

Uricosuric agents do not affect XO activity directly and as such do not limit oxidative stress. Xanthine oxidase inhibitors carry the potentially dual vasculoprotective properties of both UA reduction and reduced vascular oxidative stress. Rasburicase is typically utilised as an adjunct to cytotoxic therapy in the management of malignant disease. It is not readily administrable on a long term basis and ought not to influence the XO system activity.

Allopurinol is the XO inhibitor most frequently encountered in clinical practice, typically in the prophylaxis of gout⁴⁰¹, but also in patients with malignancy. Allopurinol is metabolised to an active metabolite, Oxypurinol which is a structural analogue of hypoxanthine and functions as a competitive agonist to inhibit XO activity^{402, 403}. It is considered to be well tolerated with few side-effects, which typically comprise rash or gastrointestinal symptoms. Idiosyncratic adverse drug reactions have been reported, albeit infrequently with an incidence of less than 1 in 1000 patients treated^{404, 405}. However, reactions may be severe and include eosinophilia, interstitial nephritis, hepatic dysfunction, vasculitis and exfoliative dermatitis. Leucopenia and thrombocytopenia are also reported. The risk of adverse reactions appears heightened in the context of renal impairment and concurrent antibiotic therapy⁴⁰⁶. More recently, an association with HLA-B5801 has been identified, which identifies a group of patients at significantly increased risk of severe reactions, including Stevens–Johnson syndrome & toxic epidermal necrolysis⁴⁰⁷. Screening for this HLA type prior to commencing treatment ought to reduce the likelihood of severe adverse reactions. In general, the adverse reaction profile is comparable to other drugs established in the primary and secondary prevention of cardiovascular disease.

There are no adequately powered clinical endpoint trials of UA lowering strategies. Three drugs known to reduce cardiovascular mortality have been shown to reduce SUA, which, hypothetically, may explain some of their beneficial effect.

Fenofibrate lowers triglyceride, total and LDL cholesterol levels and increases HDL cholesterol ⁴⁰⁸. It also reduces SUA level (via increased renal excretion) by as much as 46% ⁴⁰⁹. Losartan is an angiotensin II receptor antagonist known to reduce SUA levels by up to 30% ⁴¹⁰, via increased renal UA excretion ⁴¹¹. Nearly a third of the RR reduction seen with Losartan use in the LIFE study has been reported as potentially attributable to its effect on SUA ⁴¹². Atorvastatin has been shown to reduce SUA (by approximately 8%), even after adjustment for risk factors, including change in renal function. Each 60µmol/l reduction in SUA following Atorvastatin use was associated with a reduction in vascular event rates (HR 0.76, 95% CI 0.62-0.89) ⁴¹³.

Probenecid, a uricosuric agent which does not affect XO, has been studied in patients with heart failure. In a randomised crossover design, Probenecid 500mg bd for three weeks reduced SUA (to 0.25 mmol/l) in comparison to placebo (where it remained elevated at 0.44 mmol/l). This reduction was similar to that observed with allopurinol 300mg od but whereas Allopurinol improved endothelial function, Probenecid did not, suggesting that the mechanism of benefit of allopurinol was UA-independent ²⁹⁹.

Recent clinical research has focused on the use of the XO inhibitors allopurinol and Oxypurinol in the prevention of cardiovascular diseases. XO inhibitors lowers SUA levels, free radical production and there is evidence that the drug has a direct scavenging effect on free radicals.

Specifically with regard to stroke, allopurinol attenuates the rise in inflammatory markers seen after stroke ⁴¹⁴ and improves basal levels of cerebrovascular NO in those with diabetes ⁴¹⁵.

The results from the largest study of XO inhibitors to date were disappointing. In the OPT-CHF trial ³⁹⁸, 405 participants with heart failure were randomised to receive either Oxypurinol 600 mg or placebo for 6 months. The dose of Oxypurinol used in this trial was equivalent to only 81mg of Allopurinol, limiting interpretation of the findings. The primary endpoint was defined as a change in clinical status based upon changes in a variety of clinical parameters, including mortality and common measures of heart failure severity. There was no difference in the proportion of patients who improved or worsened between treatment groups. However, post-hoc analyses suggested that in those with elevated SUA, Oxypurinol improved clinical

status, whereas the opposite occurred in those with lower UA levels. In the Oxypurinol cohort as a whole, those who improved had significantly greater reductions in SUA levels than those who worsened. These findings are difficult to interpret. On the one-hand the post-hoc analyses suggest benefit in those with high UA levels and that the greater the fall following Oxypurinol in such patients, the better the outcome. They also raise the possibility that Oxypurinol can cause harm in some.

4.6.1. Chapter 5 aims & hypothesis

There is extensive epidemiological evidence which demonstrates an independent association between elevated SUA and risk of both CV events & mortality, including stroke disease outcomes. This evidence has given rise to the hypothesis that UA is either causally related to CV disease, or alternatively represents a surrogate measure of an alternative novel mechanism of risk; XO related oxidative stress. Discrepancies in this relationship have been observed in some populations and a counterpoint hypothesis is that UA merely represents a surrogate measure of heightened risk due to prevalence and severity of concomitant established CVRF burden.

Uric acid holds mechanistic properties which theoretically confer increased risk of atherosclerotic disease. Similarly, the enzymatic pathway responsible for UA production is a significant source of the ROS and vascular oxidative stress that are implicated in atherogenesis. Conversely, UA holds antioxidant properties which are potentially beneficial in the context of ischaemic conditions and can improve endothelial function.

Xanthine oxidase inhibition appears to be the likeliest UA lowering strategy to hold potential benefit in relation to cardiovascular disease, given its dual mechanism of action in reducing both UA and oxidative stress. There is emerging evidence in the human population relating to reduction of UA via XOI. However, there are limited studies that have examined outcomes in large samples. Several small and medium sized studies have examined the effect of pharmacological XOI on cardiovascular function, in a variety of patient populations. The reporting of positive outcomes has prompted calls for large-scale clinical trials, with definitive outcome parameters, to be conducted. However, the indication for such studies has been debated as there have also been equivocal reports in the literature.

Chapter 5 reports the detail and findings of a systematic review which evaluated the role of XOI as a potential therapeutic strategy in relation to cardiovascular disease. The aim of the study was to clarify the extent of available evidence that has evaluated the effect of XOI upon clinical or surrogate markers of cardiovascular disease and function in the human population.

Chapter 5

Xanthine oxidase inhibition for the treatment of cardiovascular disease: A systematic review and meta-analysis

5.1. Introduction

Despite widespread implementation of available preventative therapies, the incidence of cardiovascular disease remains high ⁴¹⁶. Novel therapeutic strategies are required to further reduce this disease burden.

A potential role for uric acid (UA) in the pathogenesis of cardiovascular disease has drawn attention in recent years ^{300, 417}. Post-hoc analyses of clinical trial data suggest that UA reduction may contribute to the reduced vascular risk afforded by some therapeutic agents, including angiotensin II receptor antagonists ⁴¹² and statins ⁴¹³. Conversely, short-term administration of UA has produced favourable responses in endothelial function ³⁸⁵.

Xanthine oxidase, the enzyme responsible for UA production, has been identified as a significant source of oxidative stress in the vasculature ³⁷⁸. Oxidative stress, in turn, holds a pivotal role in the pathogenesis of cardiovascular disease ³⁷¹, largely through its contribution to endothelial dysfunction. Thus, whilst a direct pathogenic role for UA in the vasculature remains unproven, its presence at elevated concentrations may indicate increased oxidative stress and consequent risk.

Several small and medium sized studies have examined the effect of pharmacological XO on cardiovascular function in a variety of patient populations. The reporting of positive outcomes has prompted calls for large-scale clinical trials, with definitive outcome parameters, to be conducted. However, the indication for such studies has been debated as there have also been equivocal reports in the literature.

Through systematic review, this study sought to clarify the extent of available evidence that has evaluated the effect of XO upon clinical or surrogate markers of cardiovascular disease and function in humans.

5.2. Methods

The “Meta-analysis of Observational Studies in Epidemiology: A Proposal for Reporting”⁴¹⁸, the “Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement”⁴¹⁹ and “The Quality of Reporting of Meta-analyses” (QUOROM) statement, were used as a framework for conduct of the review.

Studies were included that assessed effects of pharmacological XOI upon clinical or surrogate markers of cardiovascular disease / function in humans. All studies published in English, regardless of baseline characteristics, treatment dose, duration or administration method were considered. We excluded studies assessing only peri-operative or renal endpoints.

Search Method

A computer-assisted literature search was performed using the database Ovid MEDLINE (1948 - February Week 2 2011) and the database EMBASE (1980 to 2011 Week 7). Briefly, the search sought to identify all studies examining XOI in populations with established vascular disease (or vascular risk factors) or any population in which an outcome measure of vascular relevance was included. To maintain some homogeneity, studies based in populations with either chronic renal failure or surgical disease, were excluded. The search terms used were inclusive and are detailed in Table 5-1. Reference lists of all identified relevant articles and reviews along with articles notified to us by direct communication were screened to identify other potentially eligible studies.

Abstracts of all potentially relevant references were evaluated to determine suitability for full text retrieval. Retrieved studies were assessed for eligibility according to the pre-specified inclusion criteria without consideration of their results. An assessment of methodological quality of each included trial was made using the Cochrane risk of bias assessment tool and the Jadad Score⁴²⁰. This instrument assigns scores for reported randomization, blinding, and withdrawals. Non-randomized and uncontrolled studies were given a default score of 0. Two authors (PH and JD) independently conducted the search. Differences were resolved by consensus.

Data extraction

Data were extracted to a pre-defined form that included details on study methods, population, intervention, quality score and outcome measure.

Statistical Analysis

Data are reported in descriptive terms. Where an individual outcome measurement was reported in several studies (> 3), meta-analysis was planned in an attempt to combine and quantitatively evaluate the available data. Only studies including a control group and randomization (identified through application of the Cochrane risk of bias tool) were included in meta-analysis. The results for treatment effects in groups of healthy subjects, included in some studies in addition to the population of interest, were not included. Analysis was based upon summary statistics (sample size, mean value for the outcome of interest and corresponding SD), relating to completion of treatment phases. Review Manager 5 software (Cochrane library) was used to conduct combined analysis.

In the event of either clinical heterogeneity (in study population or design) or significant statistical heterogeneity being noted between studies included in combined analysis, adoption of a random effect analysis model was planned. P values were two tailed and statistical significance was set at the 0.05 level. Funnel plots were constructed for each analysis. Application of hypothesis testing with Egger's regression asymmetry test was planned in the event that the included studies in any given analysis numbered greater than ten. Simple visual assessment was planned in the case of fewer studies. In the event of either methodological or statistical heterogeneity, sensitivity analysis, with study by study omission from the combined analysis was planned.

Table 5-1: Systematic review search term details

1	vascular.m_titl.	43	hypercholesterolaemic.m_titl.
2	Cardiovascular.m_titl.	44	hypercholesterolemic.m_titl.
3	Cerebrovascular.m_titl.	45	hypercholesterolemia.m_titl.
4	Peripheral vascular.m_titl.	46	hyperlipidaemia.m_titl.
5	artery.m_titl.	47	hyperlipidemia.m_titl.
6	arterial.m_titl.	48	dyslipidaemia.m_titl.
7	Coronary.m_titl.	49	dyslipidemia.m_titl.
8	Carotid.m_titl.	50	Smokers.m_titl.
9	PAD.m_titl.	51	Smoking.m_titl.
10	PVD.m_titl.	52	endothelium.m_titl.
11	CVA.m_titl.	53	endothelial.m_titl.
12	Stroke.m_titl.	54	forearm blood flow.m_titl.
13	TIA.m_titl.	55	flow mediated dilatation.m_titl.
14	Transient ischaemic attack.m_titl.	56	oxidative stress.m_titl.
15	Transient ischemic attack.m_titl.	57	mortality.m_titl.
16	mini-stroke.m_titl.	58	morbidity.m_titl.
17	ministroke.m_titl.	59	Allopurinol.m_titl.
18	Cerebral infarct.m_titl.	60	Zyloprim.m_titl.
19	cerebral ischaemia.m_titl.	61	zyloric.m_titl.
20	cerebral ischemia.m_titl.	62	allohexal.m_titl.
21	Ischaemic heart disease.m_titl.	63	allosig.m_titl.
22	Ischemic heart disease.m_titl.	64	progout.m_titl.
23	IHD.m_titl.	65	puricos.m_titl.
24	CAD.m_titl.	66	oxypurinol.m_titl.
25	MI.m_titl.	67	Oxipurinol.m_titl.
26	myocardial infarction.m_titl.	68	Ossipurinolo.m_titl.
27	angina.m_titl.	69	Oxoallopurinol.m_titl.
28	ACS.m_titl.	70	Oxypurinolum.m_titl.
29	Cardiac.m_titl.	71	alloxanthine.m_titl.
30	Heart.m_titl.	72	Alloxanthin.m_titl.
31	Cardiomyopathy.m_titl.	73	DHPP.m_titl.
32	atheroma.m_titl.	74	Febuxostat.m_titl.
33	atherosclerosis.m_titl.	75	Adenuric.m_titl.
34	atherosclerotic.m_titl.	76	Uloric.m_titl.
35	hypertension.m_titl.	77	Tisopurine.m_titl.
36	hypertensive.m_titl.	78	Thiopurinol.m_titl.
37	blood pressure.m_titl.	79	xanthine oxidase.m_titl.
38	metabolic syndrome.m_titl.	80	xanthine oxidoreductase.m_titl.
39	diabetes.m_titl.	81	uric acid.m_titl.
40	diabetic.m_titl.	82	urate.m_titl.
41	cholesterol.m_titl.	83	hyperuricaemia.m_titl.
42	hypercholesterolaemia.m_titl.	84	hyperuricemia.m_titl.
85	33 or 32 or 21 or 7 or 26 or 17 or 2 or 1 or 18 or 30 or 16 or 44 or 55 or 27 or 25 or 28 or 57 or 40 or 20 or 14 or 49 or 24 or 10 or 31 or 35 or 11 or 53 or 48 or 42 or 22 or 46 or 13 or 23 or 29 or 6 or 50 or 39 or 36 or 3 or 51 or 9 or 58 or 41 or 12 or 47 or 15 or 52 or 38 or 8 or 4 or 34 or 56 or 45 or 37 or 43 or 19 or 54 or 5 (1329520)		
86	67 or 63 or 71 or 70 or 80 or 68 or 78 or 79 or 77 or 72 or 65 or 82 or 84 or 74 or 83 or 64 or 75 or 61 or 81 or 59 or 69 or 60 or 66 or 73 or 76 or 62		
87	85 and 86		
88	limit 87 to humans (1175 articles identified through MEDLINE, 1329 articles identified through EMBASE)		

5.3. Results

1329 citations were identified using the defined search strategy to interrogate the MEDLINE database. 1175 citations were identified using the defined search strategy to interrogate the EMBASE database. 1269 citations were excluded on the basis of title and abstract review alone. 60 citations were identified for full text retrieval. Of these, 20 were excluded on the basis of being non-human studies, surgical or chronic renal failure populations, ongoing studies, unavailable in English language (2 studies), non XOI intervention studies or review article. 40 articles that met the pre-specified inclusion and exclusion criteria in evaluating the effect of XOI on measures of vascular health were identified. Two papers each reported results of two separate modes of XOI ^{377, 421}, resulting in a total of 42 identified studies. Figure 5-1 shows details of study selection.

Study Types

Eleven studies used a parallel group, randomized controlled trial design ^{398, 414, 422-430}. Of these, three did not report randomization method ^{398, 422, 425} and one randomized inappropriately (allocation to treatment based on alternate entry into study) ⁴²⁶. Two were single blind (to the end-point assessor only) and did not utilise placebo in controls, as such appearing to constitute a prospective, randomized, open-label, blinded endpoint (PROBE) design ^{425, 426}.

Sixteen studies used a randomized controlled crossover design ^{299, 359, 415, 421, 431-442}. Method of randomization was not reported ^{299, 359, 421, 431-433, 435-438, 440, 441} in most and two again appeared to utilise a PROBE design ^{437, 438}. One publication additionally reported a second study, featuring cross over between treatment arms but lacking randomization ⁴²¹. This design was used in one further study ⁴⁴³.

Remaining studies used a prospective, open label intervention design, without control group ^{377, 444-448} or a population based cohort design ⁴⁴⁹⁻⁴⁵³. Whilst reporting a prospective open-label intervention, one publication reported a second study that featured intervention

and placebo-controlled treatment groups but lacked randomization between treatment arms³⁷⁷.

Despite the majority of randomised studies including robust design features with respect to randomisation and blinding, potential sources of bias were present in the form of either lack of complete outcome data reporting⁴²⁵ or lack of clarity as to whether all included patients had outcome data available^{422, 424, 428, 431, 433, 435, 437, 440, 442}, evidence of selective reporting^{421, 442} or lack of clarity in reporting of outcomes⁴²⁸, or some other potential source of bias^{398, 414, 415, 422, 423, 426, 427, 430, 432, 434}.

In the case of the latter, for crossover studies, lack of measurement of a pre-treatment phase baseline was evident in several studies^{299, 431, 433, 435, 437, 438, 440-442} or a single baseline measurement was performed without repeat measurement following washout prior to the second crossover treatment phase^{421, 434, 436, 439}. Several studies did not include a washout period between treatment phases^{299, 421, 431, 435-438, 440, 441}. Some parallel studies examining continuous outcome parameters also lacked baseline measurements from which to derive differences in change between groups during the study period^{424, 425, 429}.

None of the FBF response studies included a baseline. This may have been acceptable however as the outcome assessed was responsiveness to vasoactive mediators (endothelium dependent & independent).

Table 5-2 provides a summary of characteristics of the randomised controlled studies assessed according to the Cochrane risk assessment criteria. Further study details including study design, included patient population, drug used and duration of therapy are shown in table 5-3 (studies in patients with established vascular disease) and table 5-4 (studies in patients with no established vascular disease, with or without vascular risk factors).

Study Populations

Full details are included in tables 5-3 & 5-4. In addition, five studies also examined responses in healthy participants^{431, 437, 438, 442, 443}.

Figure 5-1: Details of systematic review study selection

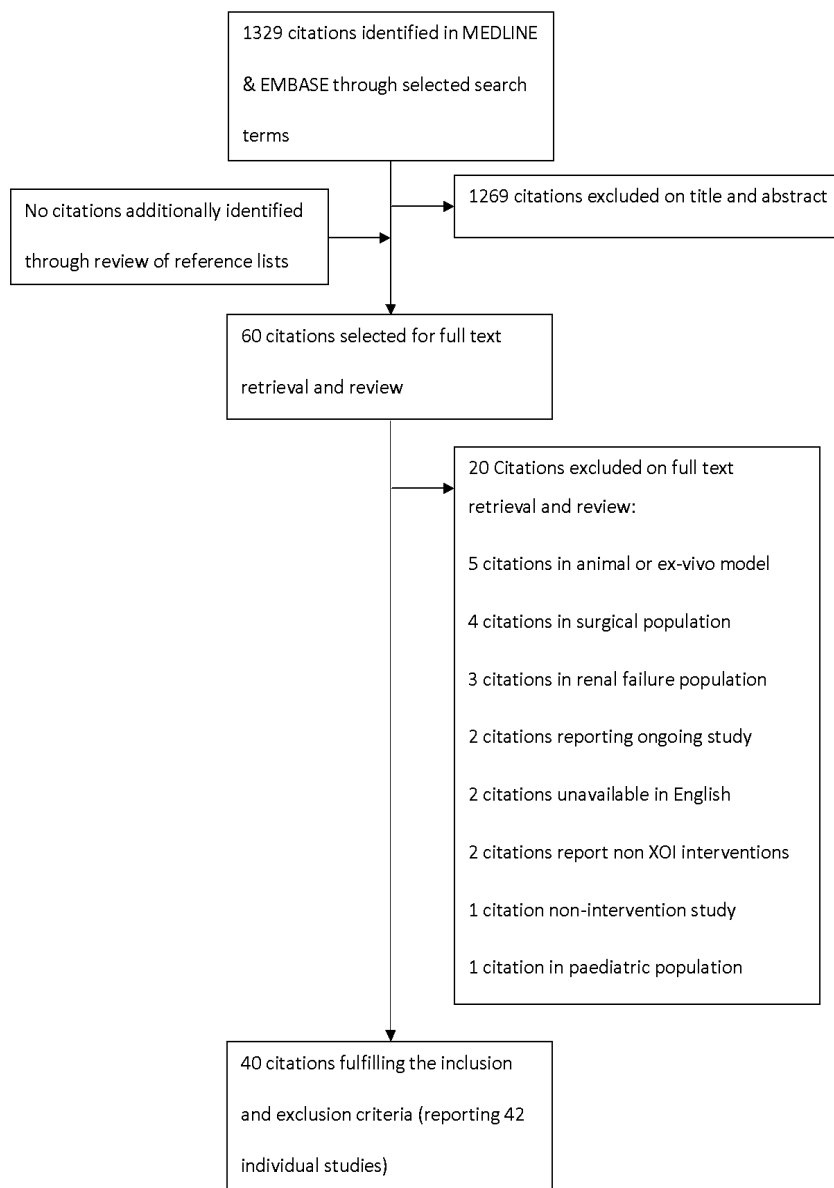


Table 5-2: Cochrane risk of bias characteristics for included studies

Study	Random sequence generation	Allocation concealment	Blinding of participants & personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Doehner 2002	Yes	Yes	Yes	Yes	Yes	Yes	No
Khan 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Rentoukas 2010	Unclear	Unclear	Unclear	Unclear	Yes	Yes	No
Muir 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hare 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yiginer 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Afshari 2004	Yes	Yes	No	Yes	Unclear	Yes	Yes
Guan 2003	Yes	Unclear	No	No	No	Yes	No
Heunks 1999	Yes	No	No	No	Yes	Yes	Yes
Parmley 1992	Yes	No	Yes	Yes	Unclear	Unclear	No
Desco 2002	Yes	Unclear	Yes	Yes	Unclear	Yes	No
Dawson 2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Delample 2008	Yes	Unclear	Yes	Yes	Unclear	Yes	No
Feig 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes
George 2006	Yes	Yes	Yes	Yes	Yes	Yes	No
Cingolani 2006	Yes	Unclear	Yes	Yes	Yes	Yes	Yes
El Solh 2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Eskurza 2006	Yes	Yes	Yes	Yes	Unclear	No	No
Gavin 2005	Yes	Unclear	Yes	Yes	Yes	Yes	No
Guthikonda 2004	Yes	Unclear	No	Yes	Yes	Yes	No
Guthikonda 2003	Yes	Unclear	No	Yes	Unclear	Yes	No
Farquharson 2002	Yes	Yes	Yes	Yes	Unclear	Yes	No
Shهاب 2001	Yes	Unclear	Yes	Yes	Yes	Yes	No
Butler 2000	Yes	Yes	Yes	Yes	Unclear	Yes	No
O'Driscoll 1999	Yes	Yes	Yes	Yes	Unclear	Yes	No
Dawson 2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Noman 2010	Yes	Yes	Yes	Yes	Yes	Yes	No

Table 5-3: Study characteristics in populations with established vascular disease

Study	Population (n)	Design	Intervention
Hare 2008	CHF (n = 405)	Parallel RCT	Oxypurinol 600mg 24 weeks
Doehner 2002	CHF (n = 14)	Crossover RCT	Allopurinol 300mg 1 week
George 2006	CHF (n = 29)	Crossover RCT	Allopurinol 300mg / 600mg 4 weeks
Cingolani 2006	CHF (n = 60)	Crossover RCT	Oxypurinol 600mg 4 weeks
Gavin 2005	CHF (n = 50)	Crossover RCT	Allopurinol 300mg 12 weeks
Farquharson 2002	CHF (n = 11)	Crossover RCT	Allopurinol 300mg 4 weeks
Shehab 2001	CHF (n = 16)	Crossover RCT	Allopurinol 300mg 8 weeks
Doehner 2002	CHF (n = 19)	Crossover, Open-label	Allopurinol Single IA infusion
Baldus 2006	CHF (n = 20)	Prospective, Open-label	Oxypurinol 400mg 1 day
Cappola 2001	CHF (n = 9)	Prospective, Open-label	Allopurinol Single IA infusion
Struthers 2002	CHF (n = 1760)	Population cohort	Allopurinol No use / 100mg / 300mg > 4 years
Wei 2009	CHF (n = 4785)	Population cohort	Allopurinol No use / <300mg / 300mg + 4.8 years (median)

Thanassoulis 2010	CHF (n = 25090)	Population cohort	Allopurinol Current use (\leq / $>$ 100mg) / No current use 2.1 years (median)
Wu 2010	CHF (n = 1152)	Population cohort	Allopurinol (Any use / No use) 14 months (median)
Parmley 2002	CAD (n = 140)	Parallel RCT	Allopurinol 600mg Duration not specified
Rentoukas 2009	CAD (n = 40)	Parallel RCT	Allopurinol 400mg loading dose then 100mg 1 month
Noman 2010	CAD (n = 60)	Crossover RCT	Allopurinol 600mg 6 weeks
Guan 2003	CAD (n = 38)	PROBE	Allopurinol 400mg 1 day
Landmesser 2007	CAD (n = 14)	Controlled study (non-randomised)	Allopurinol 600mg 4 weeks
Landmesser 2007	CAD (n = 24)	Prospective, Open-label	Oxypurinol Single IA infusion
Baldus 2005	CAD (n = 8)	Prospective, Open-label	Oxypurinol 200mg 1 day
Khan 2008	Stroke (n = 30)	Parallel RCT	Allopurinol 300mg 8 weeks
Muir 2008	Stroke (n = 50)	Parallel RCT	Allopurinol 100mg / 300mg 6 weeks
Dawson 2009	Stroke (n = 45)	Parallel RCT	Allopurinol 300mg 3 months
Table footnote: CHF – chronic heart failure, CAD – coronary artery disease, n – number, RCT – randomized controlled trial, PROBE – prospective randomized open-label blinded endpoint, mg – milligram, IA – intra-arterial.			

Table 5-4: Study characteristics in populations with vascular risk factors

Study	Population (n)	Design	Intervention
Afshari 2004	DM (n = 41)	Parallel RCT	Allopurinol 300mg 2 weeks
Desco 2002	DM (n = 12)	Parallel RCT	Allopurinol 300mg 2 weeks
Butler 2000	DM (n = 11)	Crossover RCT	Allopurinol 300mg 4 weeks
Dawson 2009	DM (n = 14)	Crossover RCT	Allopurinol 300mg 2 weeks
Feig 2008	Hypertension (n = 30)	Crossover RCT	Allopurinol 400mg 4 weeks
Cardillo 1997	Hypertension / HC (n = 20 / 20)	Crossover, Open-label	Oxypurinol Single IA infusion
O'Driscoll 1999	HC (n = 9)	Crossover RCT	Allopurinol 300mg 4 weeks
Mercuro 2004	High CV risk (n = 64)	Prospective, Open- label	Allopurinol 300mg 12 weeks
Yiginer 2008	Metabolic syndrome (n = 50)	Parallel RCT	Allopurinol 300mg 4 weeks
Guthikonda 2004	Smokers (n = 12)	PROBE (crossover)	Allopurinol 600mg 1 day
Guthikonda 2003	Smokers (n = 14)	PROBE (crossover)	Allopurinol 600mg 1 day
Heunks 1999	COPD (n = 8)	PROBE	Allopurinol 600mg 1 day
Delamplé 2008	COPD (n = 9)	Crossover RCT	Allopurinol 600mg 1 day
El Solh 2006	Obstructive sleep apnoea (n = 12)	Crossover RCT	Allopurinol 300mg 2 weeks
Spahr 2007	Liver disease (n = 17)	Prospective, Open- label	Allopurinol 400mg 10 days
Eskurza 2006	Elderly (n = 9)	Crossover RCT	Allopurinol 600mg 1 day

Kanbay 2007	Hyperuricaemia (n = 40 patients)	Prospective, Open-label	Allopurinol 300mg 12 weeks
Luk 2009	Hyperuricaemia (n = 9924)	Population cohort	Allopurinol Any use / no use
DM, diabetes mellitus; HC, hypercholesterolaemia; CV, cardiovascular; COPD, chronic obstructive pulmonary disease; n, number; RCT; randomised controlled trial; PROBE, prospective randomised open-label blinded endpoint; mg, milligram, <, less than; IA, intra-arterial.			

Study Interventions

Details of dose / 24 hours and duration of treatment are given in tables 1 & 2. Most studies utilised one dosing regimen only in design. One study used a loading dose (400mg) followed by 100mg once daily for one month⁴²⁹. The effect of variable dose (100mg / 300mg once daily)⁴¹⁴ and (300mg once daily / 300mg twice daily)²⁹⁹ was also studied. The population cohort studies compared therapy (including low or high dose) with no treatment⁴⁴⁹⁻⁴⁵³. One study did not specify treatment duration (though this appeared to have been a period of several days)⁴²⁸. Four studies examined intra-arterial treatment, via the brachial^{421, 443} or coronary arteries^{377, 454} and were conducted on a single day. The remainder involved oral treatment except two in which it was intravenous^{444, 445}.

Outcome Measures

The primary and secondary end-points used are summarised below according to broad groups of outcome measure.

Studies assessing endothelial function – Seventeen publications utilised a measure of endothelial function^{299, 377, 415, 421, 423, 427, 430, 431, 434, 435, 437, 438, 440, 442-444, 447} (nineteen studies in total as two publications each reported two separate studies)^{377, 421}.

Nine studies reported effect upon flow-mediated dilatation (FMD) of the brachial artery (as a percentage change from baseline)^{377, 421, 430, 434, 438, 442, 444, 447}, (one publication reporting FMD as an outcome for two separate studies³⁷⁷). One publication included a second study

that reported brachial artery diameter as a response to flow-mediated stimulus, without detailing the degree of dilatation ⁴²¹.

Six studies reported forearm blood flow (FBF) responses to vasoactive mediators, determined by venous occlusion plethysmography (expressed as absolute change in ml/min/100ml forearm tissue and / or as percentage change in flow relative to the non-infused control arm in each subject) ^{299, 431, 435, 437, 440, 443}. One paper reported forearm and leg blood flow responses to ischaemia ⁴²¹ and brachial artery diameter as a response to acetylcholine infusion (not expressed in terms of FBF) ⁴²¹.

Other studies examined cerebrovascular responses to NG-Monomethyl-L-Arginine (L-NMMA) infusion ⁴¹⁵, cerebrovascular reactivity to Acetazolamide ⁴²³, coronary blood flow responses ⁴⁴⁴, augmentation index (AI) ^{423, 427} and pulse wave velocity ⁴²³.

Studies assessing oxidative stress - Twelve publications examined circulating markers of oxidative stress ^{421, 422, 424-426, 430, 431, 433-435, 442, 448}. Malondialdehyde ^{422, 424, 426, 430, 431, 434, 435, 448}, Glutathione ^{424, 426, 430}, Allantoin ⁴²¹, 8-epi-prostaglandin-F2 ⁴²⁵, oxidised-LDL ⁴⁴², FRAP (total antioxidant power) ⁴²², advanced oxidation protein product (AOPP) / Isoprostanes / TAS (total antioxidant power) / Vitamin E ⁴³³ were measured.

Studies assessing cardiac outcomes - Eleven studies examined cardiac outcomes. One used a combined clinical heart failure outcome ³⁹⁸, four examined effects on cardiac function ^{425, 429, 432, 445}, one efficiency of myocardial energy use ⁴⁵⁴, one infarct extension in the ACS setting ⁴²⁸, one cardiac enzyme levels in the setting of ST-elevation MI treated with primary angioplasty and recurrent vascular events in this group during one month of follow up thereafter ⁴²⁹ and one effect on dysrhythmia counts and autonomic tone ⁴⁴¹. Four population based cohort studies examined CHF related morbidity and mortality ⁴⁵⁰⁻⁴⁵³ whilst another examined all-cause mortality ⁴⁴⁹.

Studies assessing exercise capacity - Five studies examined exercise capacity: four with walking tests, modified or standard Bruce protocol ^{398, 432, 436, 439} and one effect on muscle power and endurance ⁴³³.

Studies assessing haemodynamic measurements - Two studies examined effects on BP^{359, 446} while a further fifteen studies made reference to effects on heart rate and BP measurements^{299, 421, 426, 427, 431, 432, 435-438, 440, 443, 445, 447, 454}.

Studies assessing inflammatory / humoral indices - Several studies examined inflammatory indices^{299, 414, 415, 423, 430, 436, 439, 446, 448}, routine haematological and biochemical findings^{299, 415, 421-423, 426, 432, 436, 439, 446}, markers of haemodynamic or cardiac function^{299, 359, 436, 439} and lipids^{299, 436, 440}.

Outcome Results

Endothelial Function

Improvement in FMD following XOI was observed in eight studies^{377, 421, 430, 434, 438, 444, 447} (including both studies in one publication³⁷⁷). In one, improvement was noted only in participants stratified as hyperuricaemic⁴⁴⁷. One study which included elderly but otherwise healthy subjects found no improvement⁴⁴². No treatment effect was observed amongst healthy subjects^{438, 442}.

Five FMD studies could be combined in meta-analysis, treatment with XOI n = 75, control n = 69^{421, 430, 434, 438, 442}. Four studies were excluded because of lack of control group or randomization^{377, 444, 447} (including both studies in one publication³⁷⁷). Following treatment, patients treated with XO inhibition exhibited a 2.50 [95% confidence interval (CI), 0.15 – 4.84] higher FMD of the brachial artery, compared with control (expressed as a % change in vessel diameter, figure 5-2; figure detailing individual study data presented in Appendix B, figure B-1). The corresponding funnel plot is detailed in figure 5-3.

Improvement in FBF response to acetylcholine infusion was observed in five studies^{299, 431, 435, 437, 443} following XOI. FBF response to induced ischaemia⁴²¹ also improved. One study in hypercholesterolaemic patients failed to identify benefit⁴⁴⁰. Conversely, another showed improvement in hypercholesterolaemic but not hypertensive patients⁴⁴³. No treatment effect was seen amongst healthy subjects^{431, 437, 443}.

Five FBF studies could be combined in meta-analysis, treatment with XO n = 74, control n = 74^{299, 431, 435, 437, 440}. One study compared both 300mg once daily and 300mg twice daily with placebo and data with the lower dose was included in the combined analysis²⁹⁹. One study was excluded as it lacked randomization⁴⁴³ and one other as it examined FBF responses to induced ischaemia rather than to acetylcholine infusion⁴²¹. Following treatment, patients treated with XO inhibition exhibited a 69% [95% CI, 19 – 119%] higher FBF response to ACh, compared with control (expressed as % change in flow relative to non-infused control arm, figure 5-4; figure detailing individual study data presented in Appendix B, figure B-2). The corresponding funnel plot is detailed in figure 5-5.

The study examining brachial artery diameter responses to acetylcholine infusion and flow-mediated stimulus demonstrated improvement in both cases⁴²¹, though only amongst those who were hyperuricaemic. Coronary blood flow was augmented by allopurinol therapy⁴⁴⁴ as was bioavailability of cerebrovascular NO⁴¹⁵, though cerebrovascular reactivity to Acetazolamide was unchanged⁴²³. Augmentation index reduced in one study⁴²⁷ but was unchanged in another which also found no change in PWV⁴²³.

Figure 5-2: Forrest plot detailing end of treatment phase brachial artery FMD
(% change in vessel diameter)

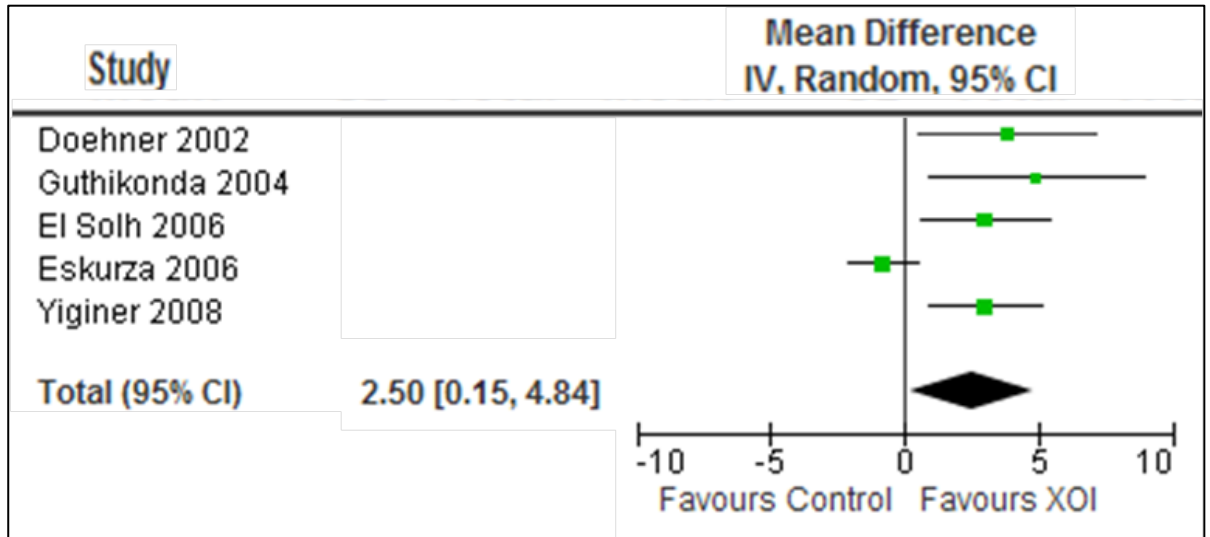


Figure 5-3: Funnel plot for brachial artery flow-mediated FMD studies

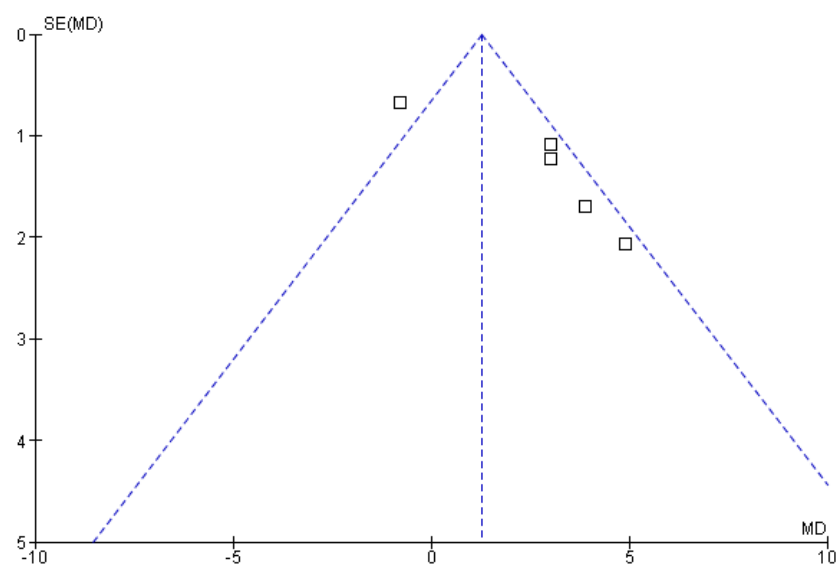


Figure 5-4: Forrest plot detailing end of treatment phase FBF response to ACh
 (% change in flow relative to non-infused control arm)

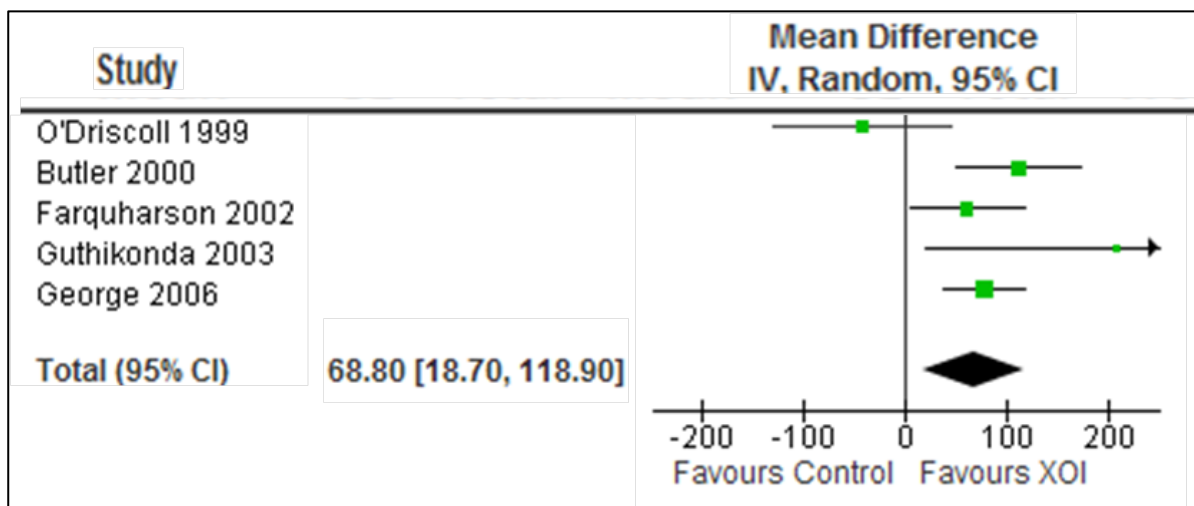
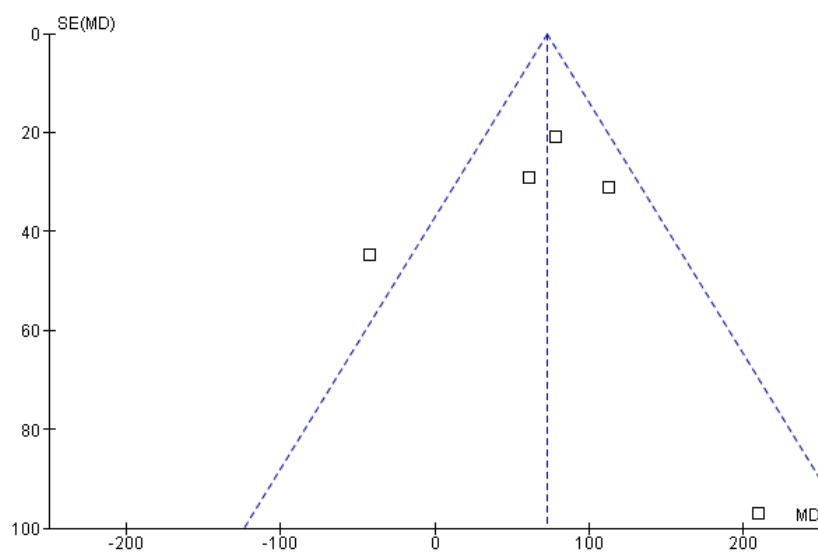


Figure 5-5: Funnel plot detailing end of treatment phase FBF response to Ach studies



Oxidative Stress Markers

Improvement was observed in ten studies^{421, 425, 426, 430, 431, 433, 433-435, 448} following XO_i, whilst four reported neutral results^{422, 424, 433, 442}, though not in all measured parameters in three cases; in one study whilst GSH levels were unchanged, GSSG and the GSSG/GSH ratio were favourably affected⁴²⁴; one study concluded no benefit despite a significant reduction in Malondialdehyde with active treatment, as similar reduction was observed with placebo⁴²²; in one study, no change in vitamin E was identified though AOPP, TAS and isoprostanes were all favourably modified⁴³³. No treatment effect was identified in healthy subjects^{431, 442} or in elderly but otherwise healthy subjects⁴⁴².

Malondialdehyde was the only oxidative stress parameter examined in more than three studies. Six Malondialdehyde studies could be combined in meta-analysis, treatment with XO_i n = 78, control n = 68^{424, 426, 430, 431, 434, 435}. One study was excluded as it included no control group⁴⁴⁸ and one as unit of reporting was unclear⁴²². Following treatment, patients treated with XO inhibition exhibited a 0.56 (95% CI, 0.26 – 0.87) lower Malondialdehyde level, compared with control (nmol/ml, figure 5-6; figure detailing individual study data presented in Appendix B, figure B-3). The corresponding funnel plot is detailed in figure 5-7.

Whilst hypothesis test were not applied to funnel plots for any of the combined analyses due to the number of studies included in each, brief inspection suggested that publication bias could not be excluded based upon the available data.

The I^2 statistic was > 50% for each of the three combined endpoint analyses, suggesting a high degree of variation across studies due to heterogeneity, rather than chance. In addition, the populations included in the combined analyses differed from one another, being defined through the presence of a specific risk factor in each case. Sensitivity analysis, with study by study omission from combined analysis are presented for FMD, FBF and oxidative stress outcome in Appendix B; tables B-1, B-2 & B-3, respectively.

Figure 5-6: Forrest plot detailing end of treatment phase circulating MDA (nmol/ml)

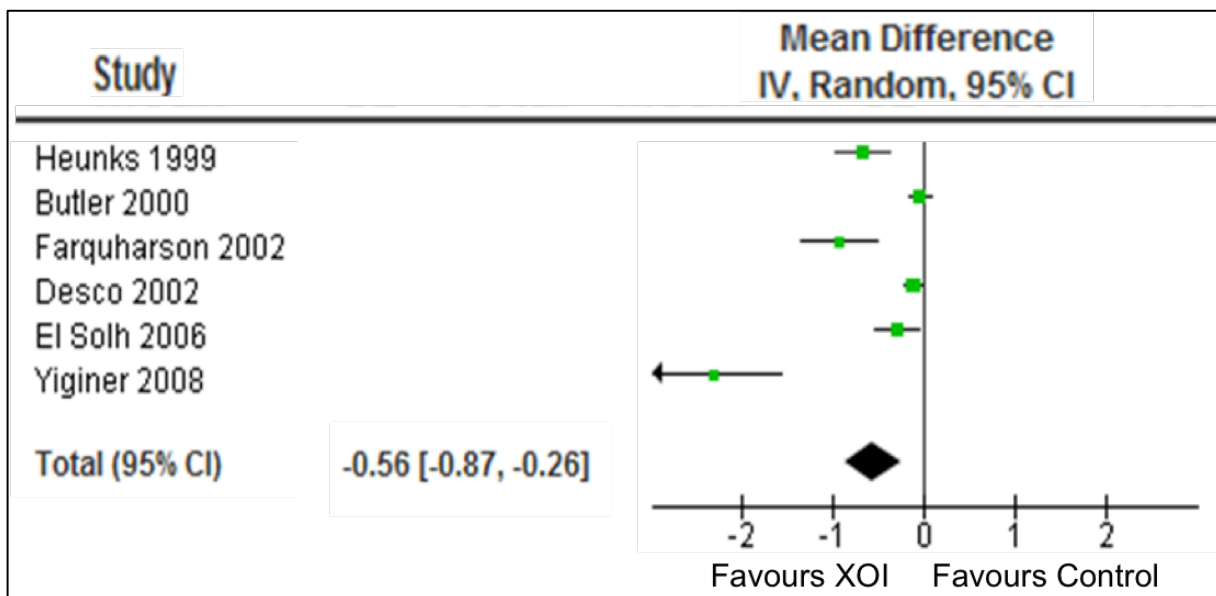
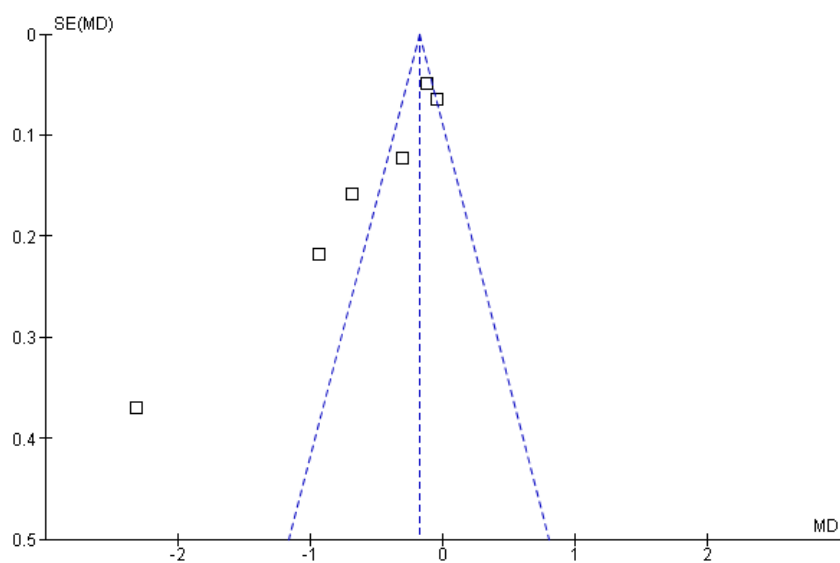


Figure 5-7: Funnel plot for serum MDA level studies



Cardiac outcomes

Four studies demonstrated improvements in cardiac physiology, including left ventricular ejection fraction (LVEF) ^{425, 432, 445}, cardiac index ⁴²⁵, end-systolic volume ⁴⁴⁵ and myocardial efficiency ⁴⁵⁴. No difference was observed with treatment upon dysrhythmia counts ⁴⁴¹ and one study found no effect upon LVEF ⁴²⁹. In the ACS setting, higher levels of “infarct extension” were seen in the allopurinol group ⁴²⁸ whilst in acute MI patients undergoing primary angioplasty, elevations in cardiac enzymes were retarded ⁴²⁹. This study, with a sample size of forty patients, reported non-significantly fewer recurrent events over one month of follow up in those treated with allopurinol (four events) than those on placebo (six events).

Two population based cohort studies identified lower heart failure related mortality in patients treated with high dose allopurinol but increased mortality in those on low dose treatment ^{450, 452}. One cohort study reported reduced heart failure combined morbidity and mortality but only amongst patients with a history of gout ⁴⁵¹. Another identified increased mortality in association with allopurinol treatment when compared with untreated patients with the lowest levels of UA ⁴⁵³. As UA level was not accounted for in the treated population, the authors drew no conclusion as to an aetiological relationship between treatment and outcome. Another cohort study reported reduced all-cause mortality amongst hyperuricaemic subjects on treatment ⁴⁴⁹. No improvement in combined heart failure morbidity & mortality was seen in a prospective study, though post-hoc analysis suggested benefit amongst hyperuricaemic patients ³⁹⁸. Heterogeneity in use of cardiac outcome measurements precluded combined meta-analysis.

Exercise capacity

Four studies reported no improvement in exercise capacity ^{398, 432, 433, 436}. One study, in patients with CAD, reported an increase in total exercise time, time to onset of anginal symptoms and time to ECG ST-segment depression ⁴³⁹. Heterogeneity in assessment method of exercise capacity precluded any combined meta-analysis.

Haemodynamic measurements

Two studies that sought to examine BP response identified a reduction with treatment^{359, 446}. One study, in the context of improved cardiac function, reported a trend to increased aortic pressure acutely following Oxypurinol administration⁴⁴⁵. No other studies making reference to routine haemodynamics reported any significant change^{299, 421, 426, 427, 431, 432, 435-438, 440, 443, 447, 454}.

Humoral / Inflammatory Indices

CRP was reduced in one study⁴⁴⁶ and was unchanged in seven others^{299, 414, 415, 423, 430, 436, 439}. Myeloperoxidase reduced in one study⁴³⁰. ICAM-1 reduced in one study⁴¹⁴ but not in another⁴¹⁵. Il-6 was unchanged^{414, 448} as was fibrinogen⁴³⁰, e-selectin and VEGF^{415, 423}.

One study observed benefit in lipid profile⁴³⁶ with no change in two others^{299, 440}. Renal parameters improved in one study⁴⁴⁶ but were unchanged in four others^{299, 421, 432, 436}. Haemoglobin reduced in one study⁴³⁶ and was unchanged in another^{426 [21]}. HbA1C was unchanged⁴²².

Reductions in Renin³⁵⁹ and BNP were reported^{436, 439} though not universally in the case of the latter²⁹⁹.

5.4. Discussion

This systematic review and meta-analysis describes the published evidence examining XOI in the human population in relation to cardiovascular health and function. This represents the only systematic review of the evidence in this area. The available data are heterogeneous in terms of study methodology, including the patient populations, treatment protocol and outcome parameters studied. There is a relative paucity of studies without potential sources of bias. However, there is a body of well-designed studies, the results of which suggest this strategy merits further study. It may represent a novel preventative strategy for cardiovascular disease.

It is important to acknowledge that there are limitations to the available evidence. There is only one large prospective randomised clinical trial in the literature. In general, prospective trials have been small, conducted in highly defined patient populations and were not universally inclusive of a control group. Moreover, when control groups were included, randomization between treatment arms and use of appropriate placebo amongst controls was sometimes lacking. Despite these flaws, there exists a body of well-designed studies that have examined a broad range of outcomes. Combined analysis was feasible for some of these outcome measures.

Improvements in measures of endothelial function, oxidative stress, cardiac function, haemodynamics and certain inflammatory indices have been demonstrated. Population based cohort studies have identified reductions in all-cause and heart failure-related mortality in association with allopurinol treatment, although, intriguingly, low dose therapy was linked with adverse outcome. The largest prospective clinical trial was conducted in CHF patients³⁹⁸. Though no overall benefit was seen in the primary outcome of the study, post-hoc analysis suggested benefit amongst patients with baseline hyperuricaemia. Limitation of benefit to participants with hyperuricaemia was reported in other populations identified in this review^{421, 447}.

The combined analysis suggests endothelial function (determined by either FBF responses or FMD) can be beneficially modified by XOI. XO activity up-regulation has been reported in pathological states such as atherosclerotic disease⁴⁵⁵. The hypothesis that impaired

endothelial function and increased XO activity are related is supported by the observation, in certain populations, that patients with hyperuricaemia appear to experience a differential response to treatment^{398, 421, 447}.

However, such benefit was not universally reported in identified studies. Conflicting results in patients with hypercholesterolaemia^{440, 443} and lack of benefit amongst hypertensives⁴⁴³ and healthy elderly participants⁴⁴² (who exhibit impaired endothelial function) were reported. No treatment effect was identified when therapy was examined in healthy participants^{431, 437, 438, 442, 443}. If we assume that XO activity is unlikely to be adversely up-regulated in such individuals, this finding may be expected.

However, lack of benefit in certain patient populations raises the possibility that up-regulation of XO activity may not be ubiquitous in all pathological states associated with endothelial dysfunction. This, combined with the differential response to treatment in relation to UA levels, may suggest that particular patient subgroups may be more, or indeed less, amenable to therapeutic XOI.

The optimal concentration of UA in man remains undefined and a better understanding of the biologic functions of UA, including its intracellular role, may prove vital if XOI is to progress as an effective therapeutic strategy in cardiovascular disease. The potential optimal level of SUA and the observed neutral results in some studies, should be considered alongside the observed dose dependent improvement in endothelial function²⁹⁹ and circulating inflammatory molecules⁴¹⁴ that were reported. Consideration of optimal dose seems essential in the design of future studies.

Whilst several studies failed to identify treatment effect on exercise capacity, only one study reported adverse outcome with XOI⁴²⁸. The observed increase in “infarct extension” amongst patients with unstable angina and MI should prompt caution. However, the redefinition of some patients as having unstable angina or a MI after recruitment into this study, in addition to the authors reporting lack of homogeneity between treatment groups in terms of concurrent therapy (including invasive revascularisation procedures), makes it difficult to draw robust conclusions from this study. Moreover, in a similar study population with acute MI, XOI was associated with retardation in cardiac enzyme elevation and no

evidence of harm ⁴²⁹. The available data suggest potential benefit of XOI in the context of acute ischemic events, in addition to the chronic effects within the arterial vasculature that have been the predominant focus of study.

Clinical relevance

It is unclear how changes in surrogate markers of vascular health may translate into clinical benefit. For the endothelial function parameters which were included in combined data analysis, both FBF responses to acetylcholine and brachial artery FMD have been identified as predictors of risk in the setting of both established vascular disease ^{456, 457} and cardiovascular risk states ^{458, 459}. As such, the endothelium and its function may be considered a potential therapeutic target.

The clinical benefits of statins and angiotensin-converting enzyme inhibitors have, in part, been attributed to their effects upon the endothelium ⁴⁶⁰. It appears, from the current study combined analysis, that XOI may produce improvement in FBF responses in the same order of effect as statin therapy ⁴⁶¹ and similar improvement in brachial artery FMD as that seen with angiotensin receptor antagonists ⁴⁶² and statins ⁴⁶³. Whilst both these drug classes clearly exert effects beyond improvement in endothelial function, it is promising that the current findings suggest a similar magnitude of beneficial effect with therapeutic XOI.

Most identified studies reported favourable XOI effects upon markers of oxidative stress. The most frequently measured of these was Malondialdehyde and the combined analysis suggested a significant, albeit modest, reduction in circulating levels that, in principle, ought to confer protection over endothelial function ³⁷¹.

Limitations of this review

Broad selection criteria were used to avoid selection bias but did not include unpublished data and did not include articles unavailable in the English language. The search strategy was systematic however data relevant to this review may have been published in a context not

identified by the search strategy. The search was based on inclusion of search terms within the title of the article. As such we may have failed to identify outcomes of interest which were referred to only within the text of the published studies. The search strategy related three concepts; population, patients with or at risk of CV disease; intervention, XO; and outcome, CV function / health parameters. Through incorporating the outcome concept, the sensitivity for identifying studies reporting outcomes which were not detailed within the search criteria terms may have been reduced.

Studies in the chronic renal failure and surgical patient populations were excluded, with a view to maintaining a degree of homogeneity in the included studies. Allopurinol is associated with adverse drug reactions, the propensity to which is increased in impaired renal function. Nevertheless, several studies have been conducted in this population with reference to cardiovascular outcomes which may potentially add to the understanding of the potential application of XO as a therapeutic strategy.

There was heterogeneity in terms of treatment duration and the population in which combined treatment effects were studied. Statistical heterogeneity (I^2) was also evident for each of the outcome measures which were included in combined analysis. Too few studies met the inclusion criteria for combined analysis to permit further statistical exploration of these differences.

Given the evidence of heterogeneity between studies varying beyond chance, a random effect model was used to estimate overall treatment effects, providing a best estimate of the intervention effect (rather than defining the average treatment effect, as in a fixed model). In only including studies in combined analysis which satisfied methodological quality criteria, the methodology minimised the risk of bias in estimating treatment effect. Other sources of bias due to population differences cannot be excluded.

The combined analysis included a small proportion of the total number of identified studies, reflecting the overall limitations of XO study designs (particularly the lack of use of a control group and randomization issues). This will have limited the ability of the random effects model to estimate the width of the distribution of intervention effects.

Though formal testing for publication bias was not applied, visual inspection of funnel plots raises this possibility. Sensitivity analysis, with sequential omission of individual studies, did not affect the observed treatment effect on MDA. However, sequential removal of the data for each study in the FMD analysis resulted in loss of overall statistical significance. The exception to this was the removal of data from Eskurza et al, which appeared responsible for the observed statistical heterogeneity. Similarly, removal of the data from Butler et al and George et al in the FBF analysis resulted in the overall treatment effect observed reducing to a non-significant trend. These findings provided incomplete reassurance that the observed results within the random effects model did not reflect exacerbating effects of either publication bias or within study-bias. The lack of benefit in the population studied by Ezkurza, which contributed to significant heterogeneity, may reflect the inclusion of elderly but otherwise healthy subjects, who may have had little to gain in terms of improvement.

Individual patient data was unavailable, with analysis based upon summary statistics included in publications, representing values obtained upon completion of treatment phases (rather than the change which occurred within each treatment group). On this basis, the observed heterogeneity between studies could not be explored further and analysis based upon individual patient characteristics was not performed.

Conclusions

This study represented the first systematic review and meta-analysis of published evidence relating to effects of XOI in relation to cardiovascular disease. A body of well-designed studies exists which have identified favourable responses in surrogate measures of vascular health following XOI. Whilst robust prospective clinical outcome data is lacking, clinical benefit has been suggested amongst patients with heart failure and with hyperuricaemia in cohort studies. There is an expanding evidence base which highlights the potential role for XOI in the management of cardiovascular disease which provides support for the conduct of large clinical trials to evaluate this strategy.

Chapter 6

Carotid intima media thickness, central blood pressure, arterial stiffness & endothelial function as surrogate markers of cardiovascular health

6.1. Rationale for use of carotid artery intima media thickness as a measure of cardiovascular function

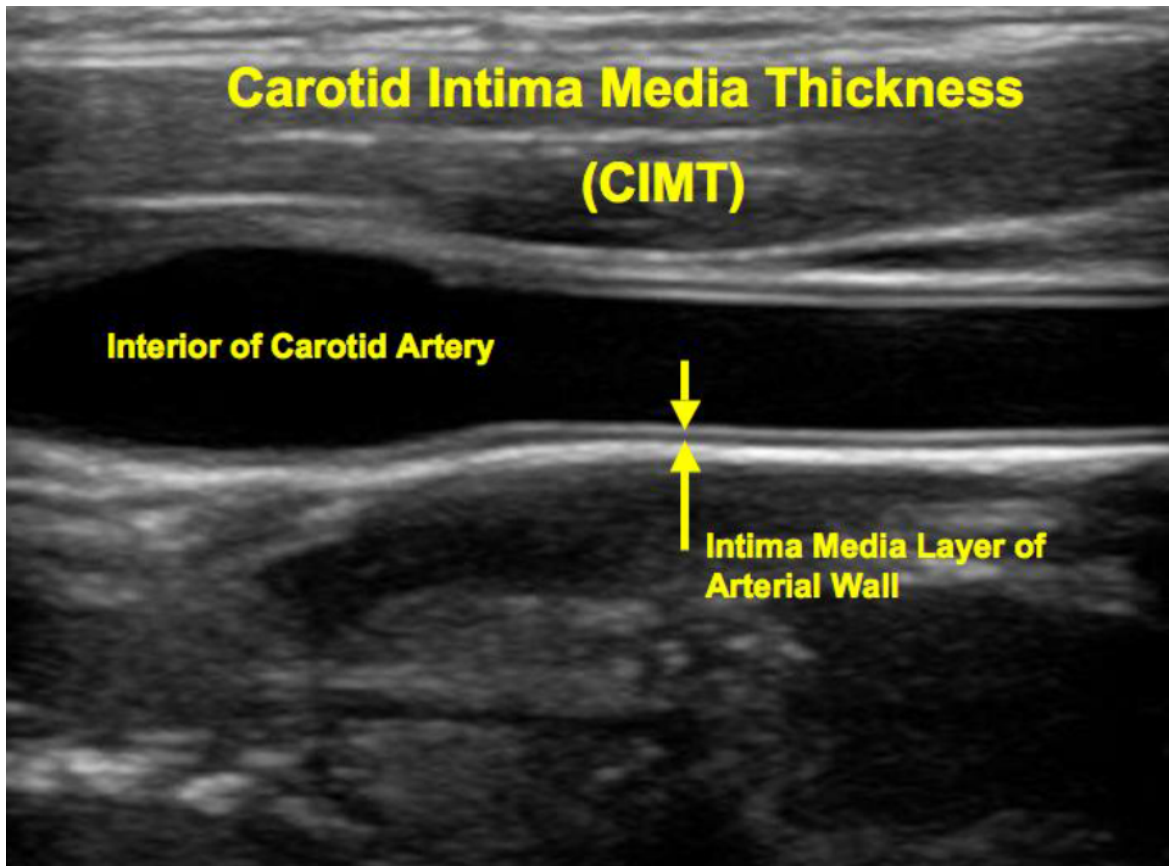
6.1.1. Carotid intima media thickness & its determinants

Atherosclerotic disease becomes clinically manifest in its advanced stages, when atherosclerotic plaque gives rise to either haemodynamic limitation and ischaemic symptoms, or plaque rupture results in an atherothrombotic event ⁴⁶⁴. However, subclinical atherosclerosis develops over years to decades ⁴⁶⁵, with the process beginning in young age, with the presence of carotid intima lesions and fatty streaks evident in the early decades of life ⁴⁶⁶. Tests for the presence of subclinical atherosclerotic disease are of interest as a potential means to stratify individual risk of cardiovascular disease and to guide use of preventative strategies. They may also hold value in monitoring response to treatment.

Intima media thickness (IMT) is defined as a composite measure of both the tunica intima and tunica media layers of the arterial wall. IMT measurement is conventionally performed at the Carotid artery (CIMT), with high resolution B mode ultrasonography ⁴⁶⁷. This readily displays the vascular wall as a regular pattern that correlates with its anatomical layers. The technique is non-invasive, well tolerated, reproducible and is considered the preferred modality for this purpose ⁴⁶⁸.

Morphological abnormalities in IMT that reflect both the initial and advanced stages in development of atherosclerotic disease are identifiable through ultrasonography, respectively manifest as IMT thickening and presence of focal atherosclerotic plaque ^{469, 470}. CIMT measurement represents a surrogate for histological assessment, correlating with atherosclerotic burden at autopsy ⁴⁶⁷.

Figure 6-1: Illustration of carotid intima media layers as visualised through B mode ultrasonography



6.1.2. Relationship between CIMT and cardiovascular risk factors

CIMT is associated with traditional risk factors for the development of atherosclerotic cardiovascular events, with numerous population based studies demonstrating a graded relationship between elevated levels of CV risk factors and increased CIMT ⁴⁷¹.

CIMT is increased amongst hypercholesterolaemic patients compared with controls ⁴⁷². In patients with familial hypercholesterolaemia, CIMT progression is accelerated, beginning at an early age ⁴⁷³. In a systematic review, a linear relationship between LDL-c and CIMT was observed ¹⁴⁷.

In the Kuopio Ischaemic Heart Disease (KIHD) Risk Factor Study, higher CIMT levels were more prevalent amongst individuals with systolic BP greater than 175mmHG (after adjustment for other risk factors) (457). The presence of hypertension is significantly

correlated with increased CIMT⁴⁷⁴ and progression in CIMT is independently associated with baseline systolic BP ($p < 0.001$ for linear trend)⁴⁷⁵.

In the ARIC population, Folsom and colleagues reported that diabetes and other surrogate risk factors for diabetes, including waist to hip ratio, were associated with carotid wall thickness⁴⁷⁶. CIMT was 0.07mm thicker amongst diabetics than non-diabetics. In the IRAS population, both diabetes and mean fasting glucose level were positively associated with common carotid artery (CCA) IMT ($p < 0.05$, following adjustment for other conventional CV risk factors)⁴⁷⁷.

Higher CIMT values were observed amongst smokers compared with non-smokers in the ARIC population, with a 0.11mm difference observed⁴⁷⁸ and both active smoking and environmental exposure to cigarette smoking were associated with greater progression in CIMT over time and the number of pack years is a powerful predictor of both baseline CIMT and progression⁴⁷⁹.

In the Carotid Atherosclerosis Progression Study (CAPS), which included 5,056 individuals aged 19 – 90 years, both baseline mean CIMT and mean ICA-CIMT measurements were each significantly correlated with baseline CVRFs, including age, male gender, hypertension, diabetes, LDL-c and smoking⁴⁷⁴. Similar independent associations with mean common CIMT were observed in the Baltimore Longitudinal Study of Aging⁴⁸⁰ and in a younger adult population. Between the ages of 20 – 90 years, mean common CIMT increases more than two-fold and in more elderly individuals, aged 60 – 80 years, CIMT increases continuously with increasing age⁴⁸¹.

CIMT also appears related to novel risk factors, including lipoprotein a⁴⁸², oxidised LDL⁴⁸³ and homocysteine⁴⁸⁴. Cardiovascular events are common amongst patients with chronic inflammatory disorders and surrogate markers of systemic inflammation are associated with CIMT, including CRP⁴⁸⁵. Genetic determinants of CIMT have been reported in linkage analysis studies⁴⁸⁶.

Several small studies have reported an association between SUA and CIMT. In 124 healthy individuals with normal range SUA, CIMT was significantly correlated with SUA concentration ($r = 0.346$, $p < 0.0001$)⁴⁸⁷, independently of other CVRFs. In an elderly Japanese population,

SUA was found to be independently associated with CIMT ⁴⁸⁸. In the CARDIA study, which included young individuals aged 18 – 30, an independent association between mean maximum common CIMT and elevated levels of baseline SUA was observed ⁴⁸⁹. In two small studies adopting a case control design in patients with hypertension, a modest but significant independent association between SUA and CIMT was observed ^{366, 490}. However, there have been few large population studies which examined the relationship between SUA and CIMT. In the Korean Multi-Rural Communities Cohort Study, which included 5,568 participants, no significant correlation was identified between SUA and a poorly defined measure of common CIMT ³⁶⁷.

6.1.3. Relationship between CIMT and atherosclerotic disease burden

In the ARIC study population, CIMT was higher amongst participants with prevalent CV disease compared to those without and prevalence of CV disease was higher amongst individuals with increased CIMT ⁴⁹¹. This finding was observed for coronary, cerebrovascular and peripheral arterial disease. An association between CIMT and both lower limb peripheral arterial disease and abdominal aortic atherosclerosis has been reported ^{481, 492, 493}. These findings provide supportive evidence for CIMT as a biomarker for systemic atherosclerotic disease.

CIMT has been associated with evidence of atherosclerotic disease burden in other arterial territories. Coronary artery calcification, measured with computed tomography scanning, is a surrogate marker for the presence of coronary atherosclerosis, an independent predictor of CHD events and a significant association between coronary artery calcification (CAC) and CIMT has been reported in both males and females ⁴⁹⁴. A graded association between CAC and CIMT was observed amongst older adults in the Rotterdam Coronary Calcification study ⁴⁹⁵ but CIMT is also significantly associated with identification of CAC in younger populations, aged 33-42 years ⁴⁹⁴.

CIMT has been associated with angiographically demonstrable coronary artery atherosclerosis in a number of populations ⁴⁹⁶⁻⁴⁹⁸. High sensitivity and specificity for CAD through CIMT measurement has been reported ⁴⁹⁹. Conversely, other groups found only

modest associations⁵⁰⁰, concluding that CIMT may not be useful as a surrogate for coronary atherosclerosis as it was neither sensitive nor specific enough to identify patients with or without significant coronary disease. Other studies reported that significant correlations were limited to CIMT measurements obtained from more distal segments of the carotid artery⁴⁹⁷. These differences are likely to reflect, at least in part, differing methodology in terms of definitions of CAD (high grade versus low grade) and in the CIMT measurement utilised. Sample size may also have limited power to identify true associations. In the Cholesterol Lowering Atherosclerosis Study, Mack and colleagues observed a significant correlation ($r=0.28$, $P=0.002$), between change in CIMT and change in angiographic coronary disease amongst 133 male patients with mild / moderate coronary stenotic disease⁵⁰¹. An association was less evident for high grade coronary lesions.

CIMT is associated with endothelial function. Amongst 2109 healthy young adults (aged 24 – 39 years), brachial artery FMD was inversely associated with IMT ($p<0.001$)⁵⁰². In this study, the number of prevalent risk factors for CV disease was correlated with IMT amongst patients with impaired FMD but not amongst those with preserved FMD, prompting the authors to conclude that endothelial dysfunction may modify the association between risk factors and atherosclerosis, with endothelial dysfunction potentially representing an early event in atherosclerosis. CIMT is also inversely associated with FMD in patients with suspected CAD⁵⁰³.

6.1.4. Relationship between CIMT and risk of subsequent cardiovascular events

Several population studies have examined the relationship between CIMT and the risk of subsequent CV events. CIMT was first reported to predict cardiovascular events in the Danish Kuopio Heart Disease Risk Factor Study, which included 1287 middle aged, male individuals. For every 0.1mm increment in CIMT, determined as maximum common CIMT, the risk of MI increased by 11% ($p<0.001$)⁵⁰⁴.

The Atherosclerosis Risk in Communities (ARIC) Study provided evidence of independent predictive value of CIMT measurement in a middle aged, asymptomatic population. This

study included 12,841 individuals aged 45 – 64 years without prior history of CHD⁵⁰⁵. The authors reported a significantly increased risk of CHD events in male individuals with CIMT \geq 1mm compared with $<$ 1mm (HR 5.07; 95% CI 3.08 – 8.36). A significant though less powerful relationship was also observed in female individuals (HR 1.85; 95% CI 1.29 – 2.69). An HR of 1.42 (95% CI, 1.24 – 1.64) per single SD increase in baseline mean common CIMT (\sim 0.19mm, adjusted for major CVRFs) was reported in males.

In a separate analysis of the ARIC population data, in 14,214 patients without prior stroke, an increased risk of incident stroke for patients with CIMT \geq 1mm compares to those with CIMT $<$ 0.6mm, in both men (HR 3.6; 95% CI 1.5 – 9.2) and women (HR 8.5; 95% CI 3.5 – 20.7) was observed⁵⁰⁶. An HR of 1.38 (95%CI, 1.16 – 1.65) per single SD increase in baseline mean common CIMT (\sim 0.19mm, adjusted for major CVRFs) was reported in males. The authors reported a graded but non-linear relationship, with the hazard increasing more rapidly at lower CIMT values.

The Rotterdam study employed a retrospective, nested case-control design to examine the relationship between baseline mean common CIMT and risk of subsequent cardiovascular events. The analysis included over 1300 individuals aged \geq 55 years, with or without prior CV disease, for whom CIMT analysis information was available⁵⁰⁷. The authors reported an increased risk of stroke (OR 1.34; 95% CI, 1.08 – 1.67) per single SD increase in baseline mean common CIMT (\sim 0.163mm, adjusted for age, sex and other CVRFs). The risk of MI was non-significantly increased (OR 1.25: 95% CI, 0.98 – 1.58) although in individuals without a prior history of cardiovascular disease, a significant association was observed⁵⁰⁸. Risk was similarly increased irrespective of sex.

The Cardiovascular Health Study provided evidence of independent predictive value of CIMT measurement in a more elderly population. This study included 4476 patients aged \geq 65 years from 4 communities in the USA, without prior history of cardiovascular disease⁵⁰⁹. During median follow-up 6.2 years, compared with patients in the lowest quintile, patients in the highest mean maximum CIMT quintile were at increased risk of CV events (RR 3.15; 95% CI, 2.19 – 4.52). The authors reported significantly increased risk for both mean maximum common CIMT (RR 1.27; 95% CI 1.17 – 1.38) per single SD increase in CIMT (0.20mm,

adjusted for conventional CVRFs) and mean maximum internal CIMT (RR 1.30; 95% CI 1.20 – 1.41) per single SD increase in CIMT (0.55mm, adjusted for conventional CVRFs).

The LILAC study reported significant independent predictive value of CIMT measurement in relation to CVM in a population of 298 individuals aged 75 and over and reported increased RR of ACM and vascular mortality during a mean 1152 days follow up for higher CIMT values⁵¹⁰.

The Carotid Atherosclerosis Progression Study (CAPS) study included 5056 individuals between 19 – 90 years old⁵¹¹. Hazard rate ratios [HRRs] per 1 SD mean CCA-IMT increase were 1.43 [95% CI: 1.35 to 1.51] for MI, 1.47 [1.35 to 1.60] for stroke, and 1.45 [1.38 to 1.52] for MI, stroke or death. CIMT was predictive of endpoints at all carotid levels. This study provided evidence of independent predictive value of CIMT across a wide age range. However, the authors reported a stronger association between CIMT and clinical endpoints for younger (HRR per 0.1mm mean common CIMT 1.34; 95% CI 1.16 – 1.55) than for older adults (HRR per 0.1mm mean common CIMT 1.10; 95% CI 1.05 – 1.15). This interaction between age and IMT prompted the authors to conclude that rather than chronological age *per se*, vascular age (determined as mean common CIMT) may be a more powerful predictor of cardiovascular risk

In all of these studies, CIMT independently predicted CV events following adjustment for age, sex and traditional CVRFs. Some notable differences in findings of these large prospective studies were observed. Some studies reported a stronger association between CIMT and CV risk for males than in females⁵⁰⁵ whilst others found no such difference⁵⁰⁷. In most populations, the relationship between CIMT and risk of either CHD or stroke events was similar. One study reported a weaker association for stroke than for CHD, though this population examined a younger population and was underpowered⁵¹¹.

Much of the prospectively collected data relating CIMT with CV outcomes has been combined in meta-analysis (meta-regression) by Lorenz and colleagues⁵¹². The adjusted (age, sex & traditional CV risk factors) HR per 1 SD difference in CCA-IMT was 1.17 (95% CI, 1.13 – 1.28) for MI and 1.23 (95% CI, 1.18 – 1.28) for stroke. This was based on inclusion of various CIMT definitions. To achieve some homogeneity, adjusted HRs per 0.1mm difference

in CIMT were calculated for mean CCA CIMT. The HR was 1.08 (95% CI, 1.04 – 1.12) for MI and 1.14 (95% CI, 1.10 – 1.18) for stroke. Despite, individual studies reporting subtle differences between the association for CHD and stroke, CIMT appears to independently predict both CHD and stroke events.

6.1.5. The relevance of carotid plaque in CIMT measurement

Atherosclerotic plaque may be identified during carotid IMT scanning, identifiable as focal thickening, with or without mineralisation and encroachment in to the arterial lumen ⁵¹³. The presence of plaque indicates the existence of focal advanced atherosclerotic disease. Measurement of plaque, both qualitatively and quantitatively, has been proposed as a potentially superior strategy to CIMT measurement for the purposes of cardiovascular risk stratification.

A graded association between CAC and carotid plaque has been observed in middle aged adults, which appeared stronger for plaque than with CIMT ^{495, 514} and CIMT bulb segment plaque is significantly correlated with angiographically demonstrable coronary atherosclerosis ⁴⁹⁷. In elderly male individuals, detection of carotid plaque lesions with B mode US, independently predicted risk of all-cause mortality ⁵¹⁵. The CAFE-CAVES study analysed carotid US imaging and categorically stratified patients according to evidence of subclinical atherosclerosis. After 10 years follow up, 8.6% of patients with increased baseline CIMT experienced incident CV events, compared with 39.28% of patients with non-stenosing focal plaque, indicating a differential increase in risk of events in the presence of carotid plaque ⁵¹⁶.

In an ischaemic stroke population, both CIMT and carotid plaque were each correlated with risk of stroke, determined as the Framingham stroke risk score. However, carotid plaque appeared to hold greater predictive value ⁵¹⁷ and CIMT was a more powerful predictor in the absence of plaque.

An analysis of the Rotterdam baseline US images for plaque and CIMT identified that both factors (Carotid plaque HR 1.83; 95% CI 1.27 – 2.62, CIMT HR 1.95; 95% CI 1.19 to 3.19), were

significant predictors of risk of incident MI, each independently of the other⁵⁰⁸. This finding suggests additive value in identification of plaque for risk stratification purposes, complimentary to measurement of CIMT.

Population studies which identified an independent association between CIMT and risk of subsequent cardiovascular events, typically incorporated focal regions of plaque (when present), within the CIMT measurement. Thus, it is possible that the positive association observed in these studies was driven by the presence of plaque disease, rather than CIMT thickening independent of the presence of plaque. This issue has been addressed in subsequent prospective studies. In 5163 Swedish middle aged individuals, both plaque and CIMT (after adjustment for the presence of carotid plaque) were each independently associated with risk of both incident stroke⁵¹⁸ and coronary events⁵¹⁹. The authors reported that a graded association between CIMT and risk was observed for both patients with and without plaque.

Overall, the presence of plaque appears to identify patients at increased risk to those without. CIMT measurement holds independent predictive value even in the context of either presence or absence of plaque lesions. On this basis, both plaque and CIMT hold value as biomarkers of atherosclerotic disease.

6.1.6. CIMT measurement in cardiovascular risk stratification

The incremental increase in risk between patients with the highest and lowest IMT values is of sufficient magnitude to potentially provide additive information to risk stratification according to conventional risk factors⁵²⁰.

Incorporation of CIMT measurements to the Framingham cardiovascular risk stratification model in the ARIC study population was found to refine its predictive power, with principal utility in the reclassification of individuals stratified as having intermediate risk of future CV events⁵²¹. This group have also reported data which suggests that complete measurement of CIMT at all levels of the carotid adds no additional value for purposes of risk stratification compared with analysis of the common segment alone⁵²².

However, such improvement in risk stratification has not been reported for all populations. In the CAPS population, incorporation of the common CIMT measurement led to reclassification of 8.1% of the population but with a net reclassification improvement of -1.41% (p=ns)⁵²³. The authors concluded that CIMT measurement did not add to existing risk stratification procedures. More recently, Polak and colleagues assessed the relationship between CIMT measurement, risk of subsequent vascular events and the ability of CIMT measurement to improve risk stratification in the Framingham Offspring Cohort Study⁵²⁴. The authors found that both mean common CIMT and mean internal CIMT were each independently predictive of subsequent vascular events but that only mean internal CIMT improved risk stratification using the Framingham risk assessment tool.

A meta-analysis of individual patient data from 14 population based cohort studies, including 45,828 individuals, found that incorporation of mean common CIMT measurement to the Framingham risk assessment tool was associated with a significant but small improvement in risk stratification for incident MI or stroke⁵²⁵. However, the overall net reclassification improvement was small at 0.8% (95% CI, 0.1 – 1.6%). Net reclassification was higher amongst patients at intermediate risk (3.6%; 95% CI 2.7 – 4.6%). The authors concluded that although significant, the magnitude of improved risk stratification through incorporation of CIMT measurement to the Framingham model was of doubtful clinical significance.

Thus, whilst holding independent predictive power for CV events, CIMT measurement incorporation to existing risk classification assessment tools only modestly enhances their predictive accuracy. Whilst commentators have therefore suggested that this limits the value of CIMT measurement for risk stratification purposes, potential as a surrogate marker for modified CV risk in clinical trials is not excluded on this basis.

6.1.7. CIMT progression and risk of cardiovascular events

Beyond the predictive value for cardiovascular risk of CIMT as a single measurement, progression in CIMT over time has also been reported to be independently predictive of cardiovascular events ⁵²⁶. In a substudy of the Cholesterol lowering atherosclerosis study, CIMT was recorded at baseline and again at 24 months. Patients were followed up for an average of 8.8 years in relation to clinical vascular events. For each 0.03mm annual increase in CIMT, the authors observed an increased RR for non-fatal MI / CHD death of 2.2 (95% CI 1.4 – 3.6), independent of coronary measures of atherosclerosis and lipid measurements.

Other epidemiological studies have identified a similar relationship. Amongst 5,028 individuals in the Multi-Ethnic Study of Atherosclerosis (MESA), CCA CIMT progression (measured in segments free of plaque) was associated with incident stroke (HR, 1.23 per 0.05 mm/year progression in CIMT; 95% CI, 1.02-1.48) ⁵²⁷.

On the basis of these findings, coupled with the predictive power of absolute CIMT measurements and additional assorted evidence identifying CIMT as a biomarker for systemic burden of atherosclerosis, CIMT progression has been utilised as a surrogate endpoint for modified CV risk in clinical trials over the last two decades ⁴⁷¹. Use of CIMT as a surrogate outcome holds the potential advantage of reducing the sample size required for clinical trials but is reliant on the assumption (or demonstration) that change in CIMT progression corresponds to change in cardiovascular risk.

6.1.8. CIMT as an outcome parameter in clinical trials

Statin therapy has the most substantial evidence base for a treatment effect on CIMT progression. In patients with CAD but moderately elevated or normal cholesterol level, CIMT was reduced by 0.014mm over an average of 4 years follow up with Pravastatin therapy compared with a 0.048mm increase with placebo ⁵²⁸. Amongst asymptomatic hypercholesterolaemic patients, CIMT was significantly reduced with treatment with Lovastatin ⁵²⁹. In a population of patients with familial hypercholesterolaemia, high dose Atorvastatin reduced CIMT by 0.031mm after 2 years whereas CIMT increased by 0.036mm

over the same period with conventional dose Simvastatin⁵³⁰. Similarly, in the ARBITER study, high dose Atorvastatin induced CIMT regression of 0.034mm within 12 months, whereas Pravastatin was associated with progression in CIMT of 0.025mm⁵³¹. In a meta-analysis of statin therapy, reduction in LDL-C was found to be strongly correlated with CIMT reduction¹⁴⁷. Each 10% reduction in LDL-C was estimated to reduce the risk of all strokes by 15.6% (95% CI, 6.7 to 23.6) and carotid IMT by 0.73% per year (95% CI, 0.27 to 1.19%).

Although lipid levels are linearly associated with both CIMT¹⁴⁷ and risk of CHD events and LDL-c represents a pathological component of the atherosclerotic process, lipid lowering therapy is not universally associated with retardation in CIMT progression⁵³². The observation that agents which have apparently failed to reduce CIMT also lack an evidence base for efficacy in reduction in clinical events, has lent support to the concept that therapeutic strategies likely to affect a reduction in endpoints require a CIMT reducing effect as a mechanistic pathway to such benefit. Statins have been shown to hold this property, whereas Ezetimibe has not, despite significantly reducing LDL-c. Several potential explanations have been proposed for this finding, including pre-treatment with statin therapy reducing the potential for additional CIMT reduction with Ezetimibe.

In addition to LDL-c related reductions in CIMT, several other pharmacological strategies have been evaluated in relation to CIMT progression. PPAR-gamma agonists⁵³³, antihypertensive agents including Amlodipine⁵³⁴, Beta-blockers⁵³⁵ ACE-Is^{536, 537} (although not in all studies⁵³³) and intensive anti-diabetic therapy⁵³⁸ have all been associated with reduced CIMT progression.

A meta-analysis has been undertaken examining treatment effects of antihypertensive agents on CIMT⁵³⁹. In patients with CAD, diabetes and hypertension, antihypertensive therapy with a variety of agents was found to be beneficial in reducing CIMT progression. The authors found some evidence of differential benefit in CIMT reduction for similar brachial artery BP lowering effects with newer type anti-hypertensive agents.

In addition to the treatment effects reported in the therapeutic studies, several non-pharmacological interventions have been assessed in relation to CIMT progression. Based on the relative contribution of risk factors to CIMT progression, multimodal lifestyle

modification has been calculated to potentially achieve a reduction in CIMT progression of 0.13mm/year⁵⁴⁰.

6.1.9. CIMT progression & modified cardiovascular risk in clinical trials

Subsequent to the initial reported epidemiological associations between CIMT progression and cardiovascular risk, conflicting evidence has emerged as to whether such a relationship exists. Clinical trial data examining both clinical endpoints and CIMT progression concurrently have, thus far, failed to find an association between CIMT progression and observed clinical events. The ELSA study evaluated Lacidipine in 2,334 hypertensive patients⁵⁴¹. The authors found that baseline CIMT was predictive of subsequent clinical events but that the progression in CIMT appeared unrelated to the risk of clinical endpoints. The authors commented that failure to identify such a relationship may have reflected the small magnitude of CIMT progression observed, relative to baseline CIMT variation.

In addition, evidence has emerged that agents which appear to have beneficial effects with respect to CIMT progression, do not confer significant benefit in terms of reduction of cardiovascular events. Niacin has been observed to significantly reduce CIMT progression compared with Ezetimibe (despite lower LDL reductions), after only 14 months treatment ($p=0.003$)⁵⁴². In addition, reduced carotid artery atherosclerotic plaque burden (determined as cross sectional carotid wall area on MRI) was observed with 12 months of Niacin therapy compared with placebo⁵⁴³.

Despite these promising effects, clinical trial data suggests that Niacin does not reduce cardiovascular events compared with placebo. The AIM-HIGH study, which included 3414 patients followed up for 36 months, was terminated prematurely due to apparent futility in a randomised comparison of Niacin with Placebo, with respect to a combined cardiovascular events endpoint (HR 1.02, 95% CI; 0.87 – 1.21) . Subsequently, the results of the HPS-2/THRIVE study, which included 25,673 patients, were presented at the American College of Cardiology Conference in 2013. Niacin was associated with no significant treatment effect for a primary endpoint of combined cardiovascular events (Risk ratio 0.96, 95% CI; 0.90 – 1.03). Although patients enrolled in these studies were already intensively managed with

cardiovascular risk reducing agents, including statin therapy, the authors concluded that despite beneficial effects on lipid profiles and surrogate outcomes, treatment with Niacin could not be recommended⁵⁴⁴.

Two meta-analyses of studies which examined CIMT progression in relation to cardiovascular risk, each failed to identify a significant association. Costanzo and colleagues used summary statistics from clinical trials to have examined interventions in relation to both CIMT progression and clinical outcomes, in a meta-regression analysis⁵⁴⁵. 41 trials, including 18,307 participants, were included. The authors reported no significant relationship between CIMT regression and CV events and concluded that induced CIMT regression does not reflect a reduction in CV risk. A separate analysis, utilising similar methodology, included data for 15,598 patients enrolled in 28 RCTs⁵⁴⁶. The authors identified a significant reduction in odds of non-fatal MI (OR 0.82; 95% CI 0.69 – 0.96) in association with CIMT regression. However heterogeneity was observed between trial outcomes. The authors of these studies suggested caution in considering CIMT as a primary outcome measure in clinical trials.

This conclusion has been debated on the basis that meta-regression represented an unsuitable methodological approach for the aims of these studies and that lack of individual patient data left open the potential for confounding factors to have influenced the findings⁵⁴⁷. Subsequently, an individual patient data meta-analysis, including 36,984 participants, assessed CV risk according to annualised CIMT progression rate (median interval between measurements of 4 years). CIMT progression was positively correlated with CV risk based on conventional CVRFs. The HR for an endpoint of combined vascular events associated with mean common CIMT progression (following adjustment for CV risk factors), was 0.98 (95% CI 0.95 – 1.01). The authors concluded that no supportive conclusion could be drawn for the use of CIMT progression as a surrogate outcome in clinical trials⁵⁴⁸.

Thus whilst serial measurements of CIMT allow the effect of interventions on CIMT progression to be assessed, there remains a lack of definitive evidence relating the parameter of “CIMT progression” to change in cardiovascular risk. Despite a lack of definitive evidence supporting the use of CIMT progression as a surrogate endpoint in clinical trials, it remains a biomarker of vascular health for research⁵⁴⁹ and is recommended in guidelines for this purpose⁵⁵⁰.

6.1.10. What is the optimal CIMT measurement methodology?

There exist various permutations of measurement and analysis from which a CIMT value may be derived. The most commonly reported analysis parameters include mean common CIMT and mean maximum CIMT and these represent the parameters recommended for reporting by current guidelines^{520, 550, 551}.

Mean common CIMT is usually derived as the arithmetic mean of multiple measurements of CIMT taken within a 10mm segment AOI, typically the 10mm segment of the CCA immediately proximal to the carotid bulb dilatation. Mean maximum CIMT is usually derived as the arithmetic mean of the maximum single point measurement of IMT at each of 12 standard carotid artery segments: measurement of both far & near wall, at three segments (common, bulb and internal), for both the right and left carotid arteries.

Mean common CIMT is a more reproducible measure than mean maximum CIMT because multiple points of measurement within an AOI are reported as a mean value. As such it may be a more suitable measurement for examining change in CIMT over time, though it is likely to be less sensitive to change than the less reproducible measurement of mean maximum CIMT. The mean common measurement is also associated with more complete technical feasibility of measurement⁴⁷¹.

Whilst these derivations of CIMT represent those most frequently reported in recommended in current guidelines, it is important to highlight that most studies reported only one of these two parameters. Moreover, methodology for the measurement of these recommended CIMT parameters has been highly variable between studies.

Mean common CIMT in some trials was derived as an arithmetic mean of a single point measurement recorded within the AOI⁵⁰⁴, rather than as the mean of multiple measurements within the specified AOI^{507, 510, 511, 518, 519}. Many studies have reported other variations in CIMT derivation, including arithmetic means of repeated AOI measurements at multiple carotid segments^{505, 506} and mean non-common segment CIMT values^{505, 506}, with or without^{505, 506} weighting based on the length of the AOI measurements available. Several studies have included measurement of far wall CIMT only^{505, 506, 511}. Mean maximum CIMT has been reported in some studies^{552, 553}, without inclusion of particular segments within the

scanning protocol in some cases, for example the carotid bulb measurements were not included in the Cardiovascular Health Study⁵⁰⁹. Idiosyncratic CIMT derivations have been reported in noteworthy studies. Irrespective of the particular CIMT parameter derivation, these studies consistently reported an independent relationship between CIMT and risk of CV events.

6.1.11. Guidelines & consensus statements regarding CIMT measurement

Given the extensive heterogeneity in the published literature relating to IMT measurement, difficulties arise in comparing the available data between populations. Homogenisation of the methodology employed to acquire data in studies assessing IMT as an outcome is clearly desirable, not only for the reasons above but also for the advantages in relation to potential meta-analysis. Consequently recommendations and consensus documents have been produced in attempts to encourage a standardised approach to measurement and harmonise future research examining IMT as an outcome measure and its use in determination of cardiovascular risk^{471, 520, 551}.

For the purposes of cardiovascular risk assessment, CIMT measurement at multiple carotid segment levels, for example with mean maximum CIMT, is superior to analysis limited to the common segment alone^{471, 520, 551}. The basis for this is that presence of plaque lesions appears to identify patients at increased risk compared to patients without plaque and the common carotid segment is typically spared from development of such focal lesions. A full inspection of the carotid may therefore better reflect systemic atherosclerotic burden⁴⁷¹. Though current guidelines recommend screening for plaque as part of IMT scanning, this pertains to the additional information provided in relation to stratifying cardiovascular risk, as opposed to inclusion as a therapeutic target or monitoring of treatment effect and some authors have recommended that mean common CIMT be reported as the measure of choice for interval monitoring⁵²⁰.

In deriving mean common CIMT, current guidelines recommend repeated CIMT measurement within a 10mm length (or shorter if full 10mm not available) of the common carotid segment immediately proximal to the carotid bulb dilatation. Measurements should

entail the far wall alone (as this is associated with greater reproducibility) and be performed from three separate angles (again proposed as likely to reduce variability and potential bias compared with single “optimal” measurements). An arithmetic mean based on these repeated measurements should be reported. In deriving mean maximum-CIMT, current guidelines recommend that 12 arterial segments be utilised in measurement. Guidelines also recommend that each of the 12 points be measured from three separate angles. Thus mean maximum-CIMT would be a mean of the maximum values identified on 36 images, with 3 images at each desired segment ^{520, 551}.

Contemporary guidelines recommend the application of semi-automated edge-detection software systems based on the “leading edge” principle for analysis of US images in measuring CIMT ⁵⁵¹. Whilst such software tend to improve reproducibility of measurement, they tend to report higher CIMT values than manual image analysis, as was utilized in many of the large epidemiological studies to examine the role of c-IMT.

6.2. Rationale for use of blood pressure pulse wave and endothelial function analysis as measures of cardiovascular function

6.2.1. Blood pressure as a risk factor for cardiovascular disease

There is a well-established aetiological relationship between BP and the risk of cardiovascular disease. Both peripheral systolic and diastolic BP are independently related to increasing risk of IHD in a linear manner⁵⁵⁴. Therapeutic reduction of BP has been demonstrated to reduce the incidence of both IHD and stroke, in proportion to the degree of BP reduction achieved

This relationship has traditionally been described in relation to the measurement of two discrete parameters, the systolic and diastolic BP, typically measured at the brachial artery⁵⁵⁴. Accordingly, the application of BP to cardiovascular disease risk stratification in clinical practice (and the decision to treat hypertension) is conventionally based upon measurement brachial artery systolic and diastolic BP⁵⁵⁵.

6.2.2. Blood pressure as a cyclic pulse wave

Blood pressure is exerted on the arterial tree as a pulse wave (BP-PW), generated by the ejection of a volume of blood from the left ventricle during systolic contraction⁵⁵⁶. Accordingly, the BP-PW propagates through the arterial tree on a cyclic basis, corresponding to each cardiac cycle. Conventional systolic and diastolic BP values, measured at the brachial artery with a sphygmomanometer, represent the maximum and minimum points, respectively, of the cyclic BP-PW (at the brachial artery)^{556, 557}.

Blood pressure is considered to exert its deleterious effect on the arterial wall through its action as a mechanical force⁵⁵⁷. The BP-PW continually exerts a range of pressures on the arterial wall. Explanation of its effect through its maximum and minimum (systolic and diastolic) levels alone, disregards pressure effects throughout the majority of the cyclic pulsation^{557, 558}, with the total pressure exerted on the arterial wall more accurately represented by the area beneath the BP pulse wave curve^{556, 558}.

These observations have given rise to interest in the potential application of more comprehensive assessment of BP-PW morphology; blood pressure pulse wave analysis (BP-PWA)⁵⁵⁸.

6.2.3. Morphology of the blood pressure pulse wave

The morphology of the BP-PW undergoes transition as it propagates through the arterial tree due to a combination of ventricular systolic ejection characteristics, arterial compliance and sites of impedance mismatch (which contribute to incident wave reflection)^{556, 558}. In addition, BP-PW morphology also differs (including in amplitude) according to modified arterial characteristics that occur with aging and in cardiovascular disease^{556, 558}.

The BP-PW is generated by left ventricular systolic contraction. An “incident” wave, the initial characteristic of which is dependent upon the form and duration of ventricular ejection, propagates from the heart throughout the arterial tree^{556, 558, 559}. At sites of impedance mismatch, typically points of arterial branching and the high resistance peripheral arterioles, reflection of the incident wave occurs⁵⁵⁹. Both the incident and reflected wave propagate through the arterial tree at a velocity in the order of metres per second. Accordingly, the morphology of the BP-PW, recorded at any site within the arterial tree, represents a composite summation of both the incident and returning reflected pressure waves^{556, 558, 559}.

Relative to central arteries, the incident wave arrives at peripheral arterial sites progressively later within the cardiac cycle. Conversely, the reflected wave arrives appears relatively early within the cardiac cycle in peripheral vessels. Thus, a peripheral artery BP-PW measurement, recorded close to the predominant site of wave reflection, will feature incident and reflected waves with a closer temporal relation to that observed centrally. Typically this results in a composite waveform in which the arrival of the reflected waveform “augments” the incident wave pressure peak in peripheral arteries, during systole. In central arteries, with relatively delayed arrival of the reflected wave, augmentation of the incident wave occurs in diastole, resulting in augmentation of coronary blood flow⁵⁵⁹.

Arteries are compliant vessels which buffer the intermittent increase in pressure produced by left ventricular contraction. A compliant vessel absorbs a greater proportion of the pressure wave energy than a rigid vessel and the pulse wave propagates less quickly^{556, 558, 559}. Both the amplitude and the velocity of the incident and reflected waves (and in turn the morphology of the composite BP-PW) are further influenced by arterial geometry and functional characteristics^{556, 559}.

Central, predominantly elastic arteries tend to exhibit greater compliance than peripheral, predominantly muscular arteries due to their relatively high elastin content in the vessel wall^{560, 561}. This function allows for pressure generated by ventricular systole to be buffered and the energy released to be accommodated, stored and then released in diastole, facilitating both diastolic coronary perfusion and smooth continuous end-organ perfusion⁵⁵⁷. Aside from their structural elastin content, functional arterial properties, including endothelium dependent smooth muscle relaxation, also contribute to arterial compliance^{562, 563}.

Peripheral arteries assume a muscular, less compliant structure, with less energy of the BP-PW absorbed by the vessel wall. Accordingly BP-PW amplitude increases peripherally within the arterial tree^{558, 564}. In addition, BP-PW velocity increases in less compliant peripheral arteries. Consequently, this further reduces the relatively small temporal difference between incident and reflected wave appearance in peripheral arteries, exaggerating the systolic augmentation at these sites⁵⁵⁸.

Thus, in peripheral arteries, with reducing compliance and increasing proximity to sites of wave reflection, the BP pulse wave systolic peak becomes relatively narrowed and sharper and the pulse pressure is amplified relative to central values. This phenomenon is referred to as pulse pressure amplification and represents a function of: 1, differential compliance between central and peripheral arteries; 2, differential time delay between incident & reflected wave merger between central and peripheral arteries^{556, 558, 559}.

6.2.4. The blood pressure pulse wave in aging and disease: arterial stiffness

“Arterial stiffness” represents loss of arterial compliance and develops as a consequence of modified structural arterial wall components (e.g. loss of elasticity associated with the cumulative effects of arterial pulsatility with aging)^{560, 561, 565}. Arterial compliance is also determined through a non-structural, NO-mediated, endothelium dependent functional component^{562, 563}, such that endothelial dysfunction may contribute to arterial stiffness. The role of endothelial dysfunction in the pathogenesis of cardiovascular disease is discussed further in chapter 6.2.7 and 6.2.8.

Loss of arterial compliance is observed with increasing age^{558, 566, 567}. In addition, arterial stiffness is also observed across a variety of CVRF states including smoking, hypertension⁵⁶⁸, renal failure⁵⁶⁹, diabetes mellitus, hypercholesterolaemia⁵⁷⁰, and established atherosclerotic conditions⁵⁷¹.

In “stiffened” arteries, the BP-PW propagates through the arterial tree at accelerated velocity and with relatively preserved amplitude, due to reduced absorption of the pressure wave⁵⁵⁸. This is associated with several adverse haemodynamic consequences: Firstly, end organ arterial beds are exposed to higher incident wave pressures due to reduced absorption of the BP-PW by compliant arteries⁵⁷². Secondly, the reflected pressure wave returns to the proximal aorta earlier within the pulse pressure cycle, during ventricular systole. This results in “augmentation” of peak systolic pressure within the proximal aorta, increased cardiac afterload and predisposition to left ventricular hypertrophy and dysfunction^{558, 559, 573}. Thirdly, earlier arrival of the reflected pressure wave results in loss of augmentation of diastolic coronary perfusion^{558, 559, 573}.

As discussed above, both BP-PW amplitude and morphology vary according to the arterial tree site⁵⁵⁶. Therefore, measurement of peripheral (typically brachial) artery systolic & diastolic BP provides only limited & indirect information relating to the burden of BP-PW effects throughout the arterial tree. Moreover, for similar extremes in BP amplitude measured peripherally, central BP-PW waveform amplitude and morphology may differ significantly in the context of arterial stiffness compared with compliant arteries^{574, 575}.

The potentially greater harm accrued through both elevated central and end-organ BP pressures associated with arterial stiffness has led to interest in potentially additive information that determination of arterial stiffness and central BP measurement might hold in relation to the risk of cardiovascular disease. If such a relationship exists, measurement of arterial stiffness and central BP, rather than brachial artery systolic BP alone, might represent useful tools in cardiovascular risk stratification. Arterial stiffness and central BP manipulation might also represent a therapeutic target.

6.2.5. Methodological approaches to blood pressure pulse wave analysis

Characteristic features of the peripheral BP pulse waveform have been described in clinical medicine for centuries, with specific modifications to that observed in young healthy individuals described in association with both ageing and pathological conditions ^{556, 558}. Graphical illustration of the pressure pulse waveform and its variations, were first described in the late 19th century by Mahomed, who reported the “sphygmograph” obtained in various patients obtained through equipment which recorded the pressure waveform of the peripheral pulse ⁵⁵⁹. Such graphical representation of the pressure pulse wave over the full cycle allows calculation of the full pressure exerted by the pulse wave on the arterial wall.

Techniques by which the peripheral artery sphygmograph may be accurately obtained have subsequently been refined, with contemporary methodology utilising the technique of “applanation tonometry” ^{558, 564, 576}. A high fidelity pressure transducer is applied to a peripheral artery site (typically the radial artery), flattening the vessel against bone (the radius), thereby eliminating tangential pressures and allowing the pulse pressure waveform within the artery to be accurately recorded ^{558, 564, 576}. Measurement of the BP-PW through applanation tonometry has been found to be reproducible ⁵⁷⁷⁻⁵⁷⁹.

Direct measurement of central BP pulse wave morphology and amplitude may be performed through aortic catheterisation. However, this is relatively impractical and associated with a risk of complication that exceeds justification for purposes of either CV risk stratification or assessment of response to therapeutic agents. However, increased understanding of the relationship between peripheral and central BP pulse waveform morphology has permitted

the development of non-invasive means of assessing arterial stiffness and central haemodynamics through peripheral BP pulse waveform analysis (BP-PWA) ^{558, 559}.

Studies have been conducted which examined both peripheral artery sphygmography and invasive central artery pressure waveforms, facilitating the calculation of transfer functions which mathematically relate the frequency components of the peripheral waveform and those of central waveform. Consequently, together with measurement of mean arterial pressure (which varies little throughout the arterial tree despite pulse pressure amplification) ⁵⁷⁴, the application of a “generalised transfer function” may be used to reconstitute the central arterial BP-PW as a derivation of the sphygmograph recorded at a peripheral artery ⁵⁸⁰. This technique has been validated in several populations ⁵⁸¹. Thus, non-invasively derived central sphygmocardiographs provide information on central haemodynamics. In addition to central BP-PW amplitude, surrogate measures of arterial stiffness, including augmentation index may be derived.

“Augmentation index” (AI) represents a ratio between the contribution made to peak systolic pressure from the reflected BP-PW and the absolute pulse pressure within the ascending aorta: it is a measure of the “contribution” that the reflected wave makes to central BP (figure 6-2). The greater the AI, the greater the magnitude of systolic pressure attributable to pulse wave reflection ⁵⁵⁶. It represents a composite measure of arterial stiffness throughout the arterial tree as it is influenced by central (e.g. aortic) stiffness (and therefore BP pulse wave velocity (BP-PWV) and wave absorption) and peripheral impedance mismatch (e.g. peripheral arteriolar tone). Augmentation index, derived through PWA has been reported as a stable measurement relatively resistant to variation in BP between recordings ⁵⁷⁷. Central (i.e. ascending aortic) BP, may differ significantly from peripheral (e.g. brachial artery) BP and may be derived through application of the generalised transfer function to applanation tonometry measurements.

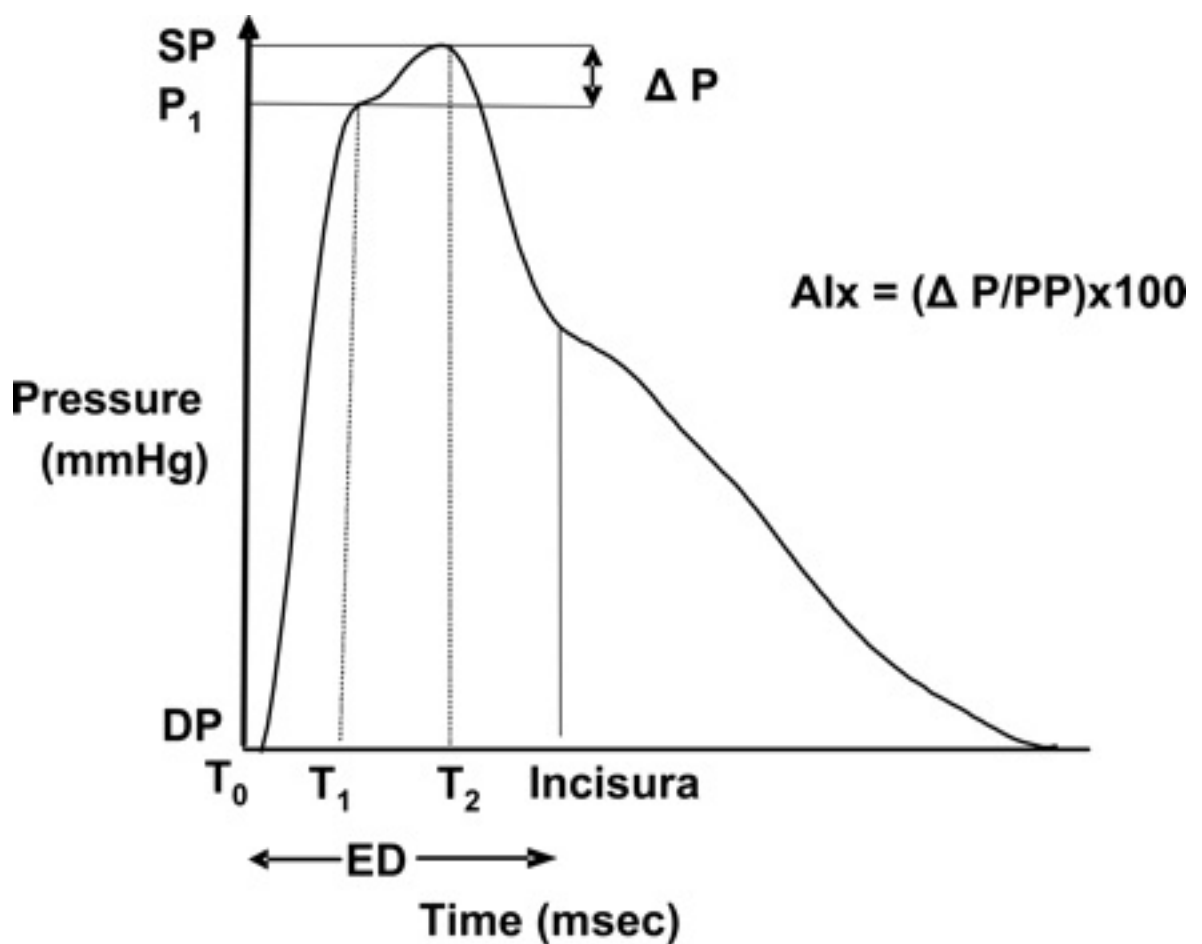
“Pulse wave velocity” is a simple measure of the time taken for the BP-PW to propagate through a given distance within the arterial tree. It may be measured directly by timing the arrival of the pressure pulse wave at two separate arterial sites in relation to the cardiac cycle. Compared with AI, PWV is a more focused measure of stiffness as the velocity within a particular arterial segment (e.g. the aorta or the brachial artery) ⁵⁵⁸, as it is less influenced by

the contribution of impedance mismatch relative to other surrogate markers of stiffness. Carotid – femoral PWV relates to aortic (central elastic arterial) stiffness. Carotid – radial PWV relates to brachial (peripheral muscular arterial) stiffness.

As AI, central BP and pulse wave velocity may all be calculated through assessment of the BP-PW, BP-PWA represents a modality by which arterial stiffness and central BP may be assessed^{556, 558, 559}. These measures have been studied in relation to subsequent risk of cardiovascular disease in a number of populations.

Figure 6-2: Illustration of central blood pressure pulse wave morphology

(reproduced from Williams et al, Circulation 2006)



6.2.6. Clinical significance of arterial stiffness and central blood pressure

Arterial stiffness, determined as various measurements, is independently associated with adverse cardiovascular outcomes amongst the general population, patients with CVRF states and those with established disease ⁵⁸². Augmentation index, based on radial artery applanation tonometry recordings, is independently predictive of all-cause mortality amongst patients with established cardiovascular disease ⁵⁸³. In addition, amongst the general population, AI predicts development of incident hypertension, independent of baseline BP ⁵⁸⁴. Though AI was found to lack independent predictive power of cardiovascular outcomes in one population ⁵⁸⁵, this was based on derivation based on recordings at the brachial artery. Pulse wave velocity independently predicts both cardiovascular endpoints ^{559, 585, 586} and mortality ^{559, 586}. Aortic BP-PWV independently predicts stroke mortality amongst hypertensive patients ⁵⁸⁷ and development of incident hypertension, independent of baseline BP ⁵⁸⁴.

When central BP measurement has been included in multivariate analysis, it has been found to hold a stronger association with risk of cardiovascular events than peripheral BP measurements ⁵⁸⁸. Indeed, brachial BP measurement loses its independent predictive value when central BP measurement is considered in some populations ⁵⁸⁹. Central arterial pulse pressure also independently predicts adverse outcome amongst patients with established vascular disease ^{590, 591} in the general population ⁵⁸⁹.

The observation that central BP may be more relevant than peripheral BP, is supported by evidence from recent large RCTs of antihypertensive therapy. Differential effects on cardiovascular outcomes have been observed for antihypertensive agents with similar peripheral BP lowering effects, raising speculation as to the mechanisms underlying the apparent superiority of newer antihypertensive agents. Subsequently, differential cardiovascular outcomes for equivalent reductions in brachial BP have been attributed to differing central BP lowering properties between agents ⁵⁹¹. In this study, central BP, determined through application tonometry at the radial artery, was preferentially reduced with ACE-Is and CCBs. Thus it appears plausible that the additive benefit of certain BP lowering drugs may be achieved through a relatively greater reduction in arterial stiffness and therefore reduced central BP.

On the basis of the epidemiological risk associations and the potential central BP related mechanism of benefit of antihypertensive therapy, arterial stiffness and central BP have been proposed as adjunctive measurements in the assessment of cardiovascular risk ⁵⁷² and as potential therapeutic targets in the prevention of cardiovascular disease ⁵⁸⁸.

6.2.7. The role of the endothelium in cardiovascular function

The endothelium was historically considered to be principally anatomical in function, separating underlying collagen and tissue from blood constituents and thereby preventing spontaneous thrombosis. Recent decades have witnessed recognition of the endothelium as an important organ system with a role in several homeostatic systems.

Through production of vasodilator and vasoconstrictor substances, the endothelium exerts extensive effects in modulating arterial tone, compliance and BP. In addition, the endothelium is involved in blood fluidity and coagulation, being responsible for production of factors which interact with platelets, the coagulation cascade and the endogenous fibrinolytic system. There is also emerging evidence that the endothelium interacts with several homeostatic systems, including glucose and lipid metabolism and appears to play a role in mediating inflammatory processes ⁴⁶⁰. Furthermore, endothelial dysfunction appears to be centrally involved in the pathogenesis of atherosclerosis ⁵⁹².

Endothelial dysfunction is a component of increasing age, risk factor states for the development of cardiovascular disease and established cardiovascular disease, including CHD, CHF and hypertension. Endothelial dysfunction has been demonstrated in subjects with an elevated lipoprotein (a) level ⁵⁹³ and in subjects with hypercholesterolaemia, an effect that appears to be mediated, at least in part, by reduced bioavailability of NO ⁵⁹⁴. Endothelial function is associated with obesity ⁵⁹⁵, models of insulin resistance such as PCOS ⁵⁹⁶, and elevated markers of systemic low grade inflammation ⁵⁹⁷. Endothelial dysfunction may represent a key feature of the metabolic syndrome.

Atherosclerosis represents an inflammatory process ⁵⁹⁸, in which endothelial dysfunction is likely to play a mediating role. Impaired endothelial function is likely to contribute to

thrombosis and endothelial dysfunction appears related to both risk of atherosclerotic plaque rupture ⁴⁶⁰. Circulating markers of endothelial activation, such as soluble ICAM-1 levels, predict the development of type 2 DM ^{599, 600}. In addition, levels are increased in conditions of endothelial damage, including following acute stroke ^{601, 602}. Elevated ICAM-1 is associated with symptomatic carotid atherosclerotic disease ⁶⁰³ and subcortical vascular disease ⁶⁰⁴.

6.2.8. Clinical significance of endothelial dysfunction

The presence of endothelial dysfunction independently predicts risk of subsequent cardiovascular events and cardiovascular mortality.

Coronary endothelial dysfunction has been shown to independently predict the occurrence of subsequent CV events ⁶⁰⁵. In addition, measurement of endothelial function in non-coronary & cerebral arterial territories holds predictive value for clinical events arising in those territories. In patients with CAD, FBF responses to ACh independently predict CV events ⁴⁵⁶. Brachial artery FMD also predicts outcomes in patients with CAD ⁴⁵⁷. These findings, coupled with the observation that brachial FMD is highly correlated with assessment of endothelial function by coronary angiography ⁶⁰⁶, suggest that endothelial dysfunction appears to be a systemic disorder. On this basis, measurement of endothelial function in non-coronary territories has been proposed as an acceptable surrogate for function in the arterial beds in which clinical events typically arise ⁴⁶⁰.

The independent predictive value of endothelium dependent vasodilatation measurement, determined as either FBF responses or FMD, extends to patient populations with risk factors but without established cardiovascular disease ^{458, 459}.

Circulating levels of intercellular adhesion molecule (ICAM-1) are an independent predictor of cardiovascular events in patients without known CVD ⁶⁰⁷. Von Willebrand factor predicts future events in patients with established CV disease ⁶⁰⁸.

Thus, measurement of endothelial function may represent a potential means by which patients at increased risk of vascular events might be identified. Measurement of endothelial

function has therefore been proposed as a tool by which to improve cardiovascular risk stratification and potentially to guide use of preventative strategies.

Most strategies of proven benefit in relation to reduced cardiovascular risk are associated with improved endothelial function. Notably, several lifestyle modifications favourably modify endothelial function. Regular aerobic exercise has been demonstrated to both prevent and reverse the age-associated decline in endothelial-mediated vasodilatation amongst a healthy, middle-aged population ⁶⁰⁹. These findings have been extended to include patients with cardiovascular disease ⁶¹⁰. Furthermore, it appears that much of this improvement in function is attributable to increased bioavailability of NO from the endothelium.

Therapeutic agents which reduce cardiovascular risk have also been found to improve endothelial function. Statin therapy improves endothelial function ⁴⁶¹, and benefit may be independent of the reduction in cholesterol associated with treatment ⁶¹¹. In addition to reduction in LDLc, statins are associated with reduced markers of systemic inflammation, which are implicated in endothelial dysfunction ⁶¹². Statin therapy has been shown to reduce ICAM-1 ⁶¹³. In addition, statins increase expression of eNOS ⁶¹⁴. Rather than plaque regression, beneficial effects on endothelial function and plaque composition are proposed as likely mediators of the benefit of statin therapy on reduction in cardiovascular risk ⁶¹⁵.

The observation that ACE-Is appear to reduce CV events beyond that expected based on the BP lowering effect has been hypothesised to be related to improvement in endothelial function ⁶¹⁶. Aside from BP reduction, ACE-Is reduce oxidative stress through decreased Angiotensin II levels, which stimulate NAD oxidase, resulting in ROS production ⁶¹⁷ and NO inactivation. ARBs also reduce endothelial markers of inflammation and oxidative stress ⁶¹⁸.

6.2.9. Methodological approaches to endothelial function assessment

Endothelial function may be measured through a variety of techniques. Nitric oxide is a potent vasodilatory molecule produced by the endothelium. Production is impaired in endothelial dysfunction. Techniques which stimulate NO production, coupled with

subsequent measurement of the vasodilatory response, provide an indirect means of determination of endothelial function: endothelium dependent vasodilatation. Several modes of NO production stimulation are available. These form the basis for the various methods of measurement of endothelium dependent vasodilatation.

Under conditions of a healthy endothelium, acetylcholine stimulates endothelial NO production, resulting in vasodilatation. Conversely, in impaired endothelial function, NO production is reduced and either an impaired vasodilatory response or vasoconstriction (due to direct effects of ACh on the media smooth muscle) is observed. Responses to infusion of vasoactive mediators permit assessment of dose response. This may be examined in the coronary arteries with angiography (necessitating coronary catheterisation) or forearm resistance vessels, using venous occlusion plethysmography (typically necessitating brachial artery catheterisation).

Shear stress associated with increased blood flow stimulates NO production. This may be measured non-invasively using vascular ultrasound of the brachial artery. Increased blood flow is produced as part of a reactive hyperaemia following transient occlusion of the brachial artery. Flow-mediated dilatation (FMD) of the brachial artery is a technique which allows serial measurement over time, given its non-invasive nature. It is highly correlated with assessment of endothelial function by coronary angiography⁶⁰⁶. Brachial artery FMD and FBF responses to infusion of vasoactive substances are less well correlated in some populations⁶¹⁹. This raises the possibility that there is differential regulation of vascular tone in conduit compared with resistance (microvascular) vessels.

Inhibition of NO synthase (e.g. with NMMLA), permits assessment of basal NO production levels, as an alternative assessment of endothelial function.

Brachial artery FMD determined through US and FBF responses to ACh determined through plethysmography are the gold standard measurements for endothelial function. However, each requires specific training and each method is dependent on operator skill and experience. FBF, whilst correlating with coronary endothelial function measurement, is invasive and unsuitable for serial measurement.

Pulse amplitude tonometry reactive hyperaemia index (PAT-RHI), represents an additional means of non-invasive measurement of endothelium dependent vasodilatation. Peripheral arterial tone measurement permits assessment of endothelial function through a standardised technique without need for extensive training and which is minimally dependent on individual operator skill and experience. As such RHI may be more readily applicable for assessment of endothelial function in clinical trials and practice ⁶²⁰. Peripheral arterial tone (PAT) measurement records changes in digital (index finger) pulse volume during reactive hyperaemia, as a surrogate marker of microvascular endothelial function. PAT measurements are performed simultaneously on both patient arms. A study arm is subjected to an induced reactive hyperaemia through inflation of a brachial artery BP cuff. The contralateral arm serves as a control, allowing correction for any systemic artefact (e.g. variation in ambient temperature). RHI is highly correlated with ultrasound determined brachial FMD ^{621, 622} and coronary endothelial function determined through ACh infusion ⁶²³. In addition, it is independently associated with numerous established CVRFs ⁶²⁴.

6.2.10. The relationship of uric acid & xanthine oxidase to arterial stiffness, blood pressure and endothelial function

As discussed in section 4.3, SUA levels are frequently elevated in the setting of hypertension and are independently predictive of development of incident hypertension ³⁵⁸. A mechanistic basis for a direct effect of UA underlying this association has been described ^{354, 355, 357} and reduction in UA through XOI has been shown to reduce brachial artery BP in certain populations ³⁵⁹. There is little data available relating UA to measures of central BP and no studies have examined effects of UA reduction on central BP.

The relationship between SUA levels and arterial stiffness has been explored in a number of studies. In the Korean Multi-Rural Communities Cohort study ³⁶⁷, Ba-PWV was increased amongst individual with hyperuricaemia, with a positive correlation and linear relationship observed in both male and female individuals. Serum UA has also been related to arterial stiffness in other populations, including post-menopausal women ³⁶⁰ and Japanese individuals ³⁶¹. In patients with prior stroke, SUA was observed to be independently

associated with measures of arterial stiffness (PWV), which was increased in those with higher SUA levels and SUA was independently associated with PWV on multivariate analysis. Moreover, in this population, treatment with Allopurinol was associated with short term evidence of reduced arterial stiffness, determined as AI^{362, 427}. Other studies have failed to identify an association between SUA and arterial stiffness and SUA^{363, 364}.

As discussed above, arterial compliance has both structural and functional components. Endothelial dysfunction is implicated in loss of arterial compliance and as discussed in Chapter 4, is itself associated with hyperuricaemia⁶²⁵. Conversely, UA is an antioxidant and administration of exogenous UA improves endothelial function³⁸⁵. Thus, UA may not represent the mediator of endothelial dysfunction but could instead be a surrogate marker for alternative mechanisms of impaired function.

Beyond UA production, XO is likely to influence endothelial function in a number of ways. There is evidence that Angiotensin II and XO activity are related^{377, 378} and XO may represent a source of NAD attributable oxidative stress³⁷⁶. Oxidative stress has been implicated as a key factor in the development of endothelial dysfunction⁴⁵⁶. Xanthine oxidase is a source of oxidative stress and consequently high levels of XO activity may confer endothelial dysfunction, with elevated SUA representing a surrogate marker for this mechanism.

Oxidative stress is implicated in the pathogenesis of arterial stiffness⁶²⁶. Allopurinol improves endothelial function, potentially through reduced oxidative stress^{299, 627} and has been shown to improve bioavailability of NO⁴¹⁵. As such, XO may well reduce arterial stiffness through affecting its functional determinants.

Allopurinol has also been shown to reduce circulating markers of endothelial activation in the short term. Allopurinol inhibits ICAM-1 expression in vitro⁶²⁸. In a small sample of patients with acute ischaemic stroke, 6 weeks treatment with Allopurinol 300mg daily was associated with a reduction in circulating ICAM-1 compared with placebo⁴¹⁴. The observed difference was driven by a rise in ICAM-1 in the placebo group at 6 weeks. This finding, rather than a reduction in the Allopurinol group and static placebo group level, may reflect attenuation of an inflammatory response by treatment.

6.3. Chapter 7 aims & hypothesis

CIMT represents a biomarker for systemic atherosclerotic burden and independently predicts risk of subsequent vascular events. In some populations, the CIMT progression over time is independently associated with modified cardiovascular risk and has been utilised as a surrogate endpoint in clinical trials evaluating preventative strategies for cardiovascular disease. Central BP, arterial stiffness and endothelial function each hold independent predictive value for risk of subsequent vascular events and are implicated in the development of atherosclerotic disease.

Although there are conflicting epidemiological associations, SUA has been independently related to each of CIMT, arterial stiffness and endothelial dysfunction. There exists a plausible mechanistic basis relating both UA and XO mediated oxidative stress to the development of atherosclerosis, arterial stiffness, elevated central BP and endothelial dysfunction.

As detailed in Chapter 5, studies examining XO inhibition have typically been of limited duration. No clinical data have been published regarding treatment effects on central BP, or measures of atherosclerosis. There is a relative paucity of data for the stroke patient population, with studies limited to brief interventions in small patient samples. The only endpoints to have been examined in this population have been cerebrovascular reactivity and AI.

In Chapter 7, the details of a study which sought to address this evidence gap are detailed. A randomised double blind placebo controlled study, concerning the effect of one years' treatment with Allopurinol 300mg daily, in a population with recent ischaemic stroke or TIA, was performed, with the hypothesis that treatment with allopurinol would reduce CIMT progression, arterial stiffness and in turn central BP and would improve endothelial function. This study would demonstrate feasibility of CIMT measurement in the local population and provide Pilot data to inform the design of a larger future study examining this endpoint.

Chapter 7

The effect of allopurinol on carotid intima-media thickness, arterial haemodynamics and endothelial function in patients with recent ischaemic stroke and TIA: A randomised controlled trial

7.1. Introduction

Despite secondary preventative measures, recurrent stroke events remain common; approximately 13% of participants suffered recurrent stroke in recent secondary preventative trials¹¹⁵ and 40% of those with TIA experienced recurrent cardiovascular events during long-term follow up⁶²⁹. Novel strategies are needed to reduce this burden.

Elevated SUA is associated with increased risk of cardiovascular disease³⁰⁰ and adverse outcomes following ischaemic stroke^{330, 394}. Allopurinol reduces SUA through inhibition of the xanthine oxidoreductase (XO) enzymatic system, which is responsible for the final steps in purine metabolism. Further, XO inhibition reduces the ROS formed through action of the enzyme and may thus reduce vascular oxidative stress, which is implicated in atherogenesis³⁷⁶. Allopurinol may therefore provide benefits in addition to or independent of its effects on UA²⁹⁹.

Systematic review and meta-analysis of studies examining XO inhibition has demonstrated beneficial effects on endothelial function and other surrogate measures of cardiovascular function⁶²⁷. Further, following stroke, allopurinol has been reported to reduce both arterial stiffness⁴²⁷ and markers of inflammation⁴¹⁴ and also increases NO bioavailability in patients with type 2 diabetes⁴¹⁵. More recently, treatment with high dose allopurinol was found to induce regression in left ventricular hypertrophy⁶³⁰ and improve exercise capacity in patients with CAD⁴³⁹. However, studies examining XO inhibition have typically been of limited duration and there are no prolonged studies in the stroke patient population, or on measures of atherosclerosis.

We sought to address this evidence gap by performing a randomised double blind placebo controlled study with a one year treatment duration, which examined the effect of allopurinol 300mg daily on change in central BP, arterial stiffness, endothelial function and circulating markers of low grade inflammation and also provided pilot data concerning the effect of allopurinol on carotid intima-media thickness progression.

7.2. Methods

Study design

This study was a randomised double blind, placebo controlled trial, comparing allopurinol 300mg once daily with matched placebo in an adult population with recent ischaemic stroke or TIA. Patients were followed up for 12 months and underwent repeated measurement of carotid artery intima media thickness (CIMT), arterial haemodynamics and endothelial function.

Study Population

Patients aged over 18 years, both males and females, with ischaemic stroke or TIA, within the past year, were eligible for inclusion. Ischaemic stroke and TIA (ICD Classification Code I63.0-9 and G45.0-1) were each defined as suggestive clinical features which can be classified according to the Oxfordshire Community Stroke Project classification and which had a presumed vascular occlusive cause. Principal exclusion criteria were >70% extra-cranial ICA stenosis, significant co-morbidity likely to cause death within 12 months and either indication for, or contra-indication to (including estimated glomerular filtration rate < 50 ml/min), administration of allopurinol. Exclusion criteria were chosen to minimise the potential risks of allopurinol treatment and to ensure carotid IMT measurements were not affected by intrinsic carotid disease. Full details of inclusion & exclusion criteria are detailed in table 7-1.

Patients were identified during admission or out-patient attendance at the Acute Stroke Unit at the Western Infirmary, Glasgow. All participants were provided with a study patient information sheet, given the opportunity to ask questions and provided written informed consent to participate. The study was approved by the West Medical research ethics committee (reference 08/S0709/87) and was registered in the ISRCTN database (ISRCTN 11970568).

Table 7-1: Principal eligibility criteria

Inclusion Criteria	<ol style="list-style-type: none"> 1. Ischaemic Stroke (including TIA, where symptoms last less than 24 hours). 2. Brain imaging not suggestive of an alternative diagnosis. 3. Randomisation within one year of ictus.
Exclusion Criteria	<ol style="list-style-type: none"> 1. >70% extra-cranial internal carotid artery stenosis. 2. Significant co-morbidity or frailty likely to cause death within 12 months or likely to make adherence to study protocol difficult for participant. 3. Contra-indication to or indication for administration of allopurinol (as detailed in Summary of Product Characteristics). 4. Concurrent azathioprine or 6-mercaptopurine therapy. 5. Significant hepatic impairment (defined as serum bilirubin, AST or ALT greater than three times upper limit of normal (ULN)). 6. Estimated Glomerular Filtration Rate < 50 mls/min 7. Cognitive impairment deemed sufficient to compromise capacity to consent or to comply with the protocol. 8. Women of childbearing potential. 9. Prisoners.

Sample size

A sample size of 40 participants per group was studied. This sample provided 80% power to detect a 6 mmHg difference in central BP ⁴²⁷ (assumed SD 10 mmHg) and 80% and 90% power to replicate in prolonged follow up, respectively, previously reported change in augmentation index (REF Khan 2008) and circulating markers of vascular inflammation (Muir 2008 REF) following Allopurinol therapy after stroke. The primary study endpoint was change in CIMT at 12 months. Based on a mean annualised CIMT progression rate of 0.0176 mm (95% CI 0.0149-0.023, SD 0.05), mean baseline CIMT of approximately 1 mm [13,14] and

a treatment effect approximately half that observed with Statin therapy [14], 253 participants in each treatment group, followed for 2 years, would provide 80% power to detect a 0.03 mm difference in IMT progression (alpha of 0.05). This study lacked power to exclude a potentially meaningful treatment effect for this endpoint and represented a pilot study, designed to allow us to confirm these assumptions for a larger CIMT trial and provide preliminary data whilst specifically addressing important pre-specified secondary endpoints."

Randomisation, study intervention & masking

Enrolled participants were randomised, via an interactive voice response system (Robertson Centre for Biostatistics, University of Glasgow), to receive either 300mg of allopurinol orally once daily or matched placebo, on a 1:1 basis. Investigators and patients were blinded to treatment allocation. Allopurinol tablets were manufactured and over encapsulated by Bilcare Ltd. Dosing began on the day following baseline assessment and randomisation and continued for one year. Concordance with therapy was assessed by questioning and pill counts. Investigators were blinded to SUA levels to avoid potential inference of study group allocation. The randomisation code was not broken until all follow up was complete and all data were prepared for analysis.

Study follow up visits and endpoint data collection

All study procedures were performed in the Acute Stroke Unit of the Western Infirmary, Glasgow. Patients attended study visits at baseline (randomisation), 1, 3, 6 & 12 months. Patients attended following an overnight fast and were asked to avoid caffeine, tobacco & alcohol for the preceding 12 hours. At each visit a clinical examination including brachial artery BP was performed, together with blood sampling and adverse event review. Brachial artery BP measurements were performed [using a semi-automated sphygmomanometer (Critikon DINAMAP)]. Following 15-minutes supine rest, three BP measurements were taken at 1-minute intervals and the mean of these was recorded. Blood testing included routine haematological and biochemical parameters, including full blood count, urea & electrolytes,

liver function tests and uric acid. Venepuncture was performed with a 19G (green needle) vacutainer system from the antecubital fossa (where possible). An EDTA tube, serum gel tube and fluoride/oxalate tube were collected (~ 8 mls in total) at each study visit, including the safety visits. Samples were sent directly to the local NHS laboratory for analysis. All blood results were reviewed within 48 hours of being taken by an investigator if abnormal.

All study visit data was recorded in the first instance to a paper based CRF, which was stored in a secure fire proof cabinet in the Western Infirmary Stroke Unit. During the course of the study, a web based electronic CRF was developed in conjunction with the Robertson Centre for Biostatistics (RCB), University of Glasgow. Data from the paper based CRFs was subsequently transcribed to the electronic system once this completed construction, was tested and subsequently became live.

7.2.1. CIMT measurement

Carotid artery intima-media thickness (CIMT) ultrasound (US) scanning protocol

A 7.5MHz Zonare Z.one Smart Cart annular array ultrasound system and L8-3 US probe (Zonare Medical Systems, CA USA) were used for carotid US CIMT image acquisition. Longitudinal carotid views were captured using B Mode ultrasound. This technique permits measurement of IMT across an AOI, for example the distal 10mm of the CCA. The maximum IMT within the AOI and the mean IMT for the length of the AOI may then be measured during offline analysis. This affords additional information and greater reproducibility of measurement than M mode US, which permits measurement of CIMT at only a single point within the vessel ⁵²⁰.

The scanning protocol used was consistent with that recommended in contemporary guidelines, at the time of the study protocol approval ^{468, 471, 520} and subsequently ⁵⁵¹, to permit derivation of both mean common CIMT and mean maximum CIMT measurements. IMT measurement was performed according to the same protocol employed in the PERFORM study, which included CIMT as a surrogate measure of outcome ⁶³¹. All IMT scans were performed by an experienced sonographer (KS), blinded to study treatment allocation, with

recent accredited experience in CIMT measurement during the conduct of the PERFORM study.

Images delineating CIMT were recorded within three separate AOIs: CCA (10mm length proximal to carotid bulb); carotid bulb (10mm length proximal to flow divider); internal carotid, 10mm distal to flow divider^{468, 471, 520}. For each of these AOIs, ultrasound images were captured at each of three pre-specified angles, encompassing a 60 degree arc: 180°, 150° and 120° (right carotid) and 180°, 210° and 240° (left carotid). A Meijer carotid arc was used to standardise the acquisition of images. Images were obtained to include both the far and near wall of the artery for each angle. Images were recorded for both the right and left carotid arteries. Thus, CIMT was imaged at a total of 12 sites within the right and left carotid arteries and from 3 separate angles at each of these sites, allowing up to 36 points of IMT measurement.

Several seconds of IMT images were recorded with the Zonare system. A still image synchronised with ventricular systole was then saved in digital format (DICOM) by the US device for each CIMT point of measurement. The study image was identified by the date of examination, participant study number and study visit month number. Each image was additionally labelled according to the arterial site being imaged and the depth of US measurement.

Carotid artery intima-media thickness (CIMT) ultrasound (US) off-line image analysis

The CIMT US image DICOM files acquired were exported to an encrypted domain on a secure NHS computer in the Acute Stroke Unit at the Glasgow Western Infirmary Hospital. Files were then converted to TIFF format to permit off line image analysis, as recommended for the purposes of deriving CIMT measurement to improve precision and reproducibility⁴⁷¹.

Many of the large epidemiological studies which reported an association between CIMT and CV outcomes employed manual reading techniques for CIMT measurement. Subsequently, the use of automated and semi-automated edge detection software has facilitated wider application of CIMT measurement as it reduces the time required to analyse individual

scans^{520, 551}. Moreover, precision and reproducibility of CIMT measurement are both demonstrably improved with this approach⁶³²⁻⁶³⁴. Current guidelines reflect this and recommend the use of semi-automated edge detection system that permit the reader to edit tracked borders if the application of the software algorithm to a particular image provides suboptimal border identification^{520, 551}.

Image-Pro Plus, Version 6.2, software (Media Cybernetics, USA) was used for CIMT US image analysis. This software has previously been used in large RCTs which included CIMT as an outcome measure⁵³³.

US images were recorded at a measurement depth of either 4 cm or 5 cm (according to individual patient arterial topography). The Image-Pro software was calibrated to accommodate either TIFF file resolution, with the separate calibrations utilized for CIMT measurement according to the depth of the US image acquired. The level of pixelar resolution (0.0789 mm / pixel & 0.0985 mm / pixel for 4 cm 5cm depth US images, respectively), compares favourably with that recommended in guidelines and reported in many of the large epidemiological studies⁵²⁰.

Images were reviewed for technical adequacy for CIMT measurement. Provided the available image displayed the vascular wall as a regular pattern that correlates with arterial anatomical layers, a CIMT measurement was recorded. CIMT measurement was based on the 10 mm AOIs as defined above. If vessel tortuosity prevented a full 10mm measurement, CIMT was measured for the maximum available length of the particular AOI.

Scans were analysed by batch reading (comprising ten scans per batch), in a randomised order. This approach was adopted to avoid theoretical change over time in CIMT estimates due to adapted reading technique⁴⁷¹.

Blood-intima and media-adventitia boundaries were marked with a calliper and semi-automated analysis of the CIMT was then performed using the leading edge principle.

For each AOI, callipers were applied at the protocol defined AOI limiting boundary to the leading edge of each of the four interfaces of interest: adventitia – media (far wall); intima – lumen (far wall); lumen – intima (near wall); media – adventitia (near wall). Based on the

leading edge principle, the auto-edging software delineated (based on an algorithm identifying the steepest point of inflection on a luminescence scale applied to these boundaries), the boundaries between the tissue planes of interest for the AOI. The image reader had the opportunity to manually edit the delineated boundary if the automated measurement clearly deviated from the interface boundary (typically due to artefact acoustic shadowing within the US image).

When observed within the AOI, carotid plaque was included in the delineated boundary, in keeping with current recommendations⁵²⁰. This afforded the opportunity to describe the proportion of patients with carotid plaque based on absolute measurements and to perform statistical analysis both with inclusion of plaque as part of the IMT continuous variable, as recommended by some⁵²⁰ and with these measurements excluded based on absolute measurements, as suggested by others⁴⁶⁸.

Following boundary delineation, the software automatically calculated both the maximum distance between interfaces and the mean distance between interfaces for the available length of the AOI (up to 10mm). Accordingly, both a maximum CIMT and a mean CIMT was generated for each AOI (up to 36 measurements for each in total). The length of the AOI for which the mean CIMT was calculated was also recorded, to permit calculation of weighted means in the event of the AOI being < 10mm for any points of measurement.

Mean and maximum CIMT measurements, together with length of the AOI were automatically exported by the Image-Pro Plus software to a Microsoft excel file, labelled with the patient subject number and visit month number. These files were then transferred to the Robertson Centre for Biostatistics for storage and subsequent statistical analysis.

CIMT endpoint derivation

The mean maximum CIMT was derived as the arithmetic mean of the maximum IMT value measured at each of the potential 36 points of CIMT measurement. I.e. a composite measure based on CIMT measurements at each of common, bulb and internal artery sites.

This definition is consistent with current guidelines for the derivation of mean maximum CIMT^{471, 520, 551}.

The mean common CIMT was calculated as a weighted mean, based on the measurements obtained from both the right and left common carotid AOIs at each of the three different measurement angles. Weighting was according to the length of common carotid IMT segment available for each of the specified AOI measurements (up to 100 mm of the common segment proximal to the carotid bulb). In keeping with recent guidelines, mean common CIMT derivation was based on measurement of the far wall only^{520, 550, 551}. Mean maximum and mean common CIMT derivation was performed by statisticians at RCB, University of Glasgow (HM, AM).

7.2.2. BP-PWA measurement

Blood pressure pulse wave analysis scanning protocol

Patients attended for BP-PWA following an overnight fast and were asked to avoid caffeine, tobacco & alcohol for the preceding 12 hours. Following 15-minutes supine rest, three brachial BP measurements were taken at 1-minute intervals, using a semi-automated sphygmomanometer (Critikon DINAMAP) and the mean of these was recorded.

BP-PWA was performed using the Sphygmocor® apparatus (PWV Medical, Sydney Australia). This utilizes applanation tonometry, a non-invasive technique permitting high-fidelity analysis of peripheral and central pulse waves^{564, 576}. A hand-held tonometer (Millar instruments, Houston USA) is applied to a peripheral (radial) artery site, flattening the wall of the artery. This eliminates tangential pressures and allows the pressure waveform within the radial artery to be recorded by the tonometer piezo-resistive pressure transducer (Miller Instruments, Houston USA)^{564, 576}. Three separate samples of right radial artery BP pulse waveforms, each of 10 seconds duration, were obtained using the Sphygmocor tonometer apparatus.

The Sphygmocor software automatically applies a validated generalized transfer function to the obtained radial waveform, facilitating derivation of the central aortic BP pulse

waveform⁵⁷⁶. The brachial artery BP was utilized for the purpose of radial artery BP calibration. Analysis of the central BP waveform permits non-invasive determination of central aortic BP pulse (based on wave amplitude) and arterial stiffness, including AI (based on wave characteristics including contour, timing & augmentation of reflected BP pulse waves)⁵⁷⁶. Augmentation index was calculated as the ratio of difference between the early & late central systolic pressure peaks to the central pulse pressure. This method of performing PWA has been found to be reproducible in a variety of populations⁵⁷⁹ and is an accepted technique for the measurement of arterial stiffness⁶³⁵.

For each patient study visit, BP-PWA measurements were performed in triplicate. These measurement data were entered to a paper CRF and subsequently transcribed to the electronic CRF and stored by the RCB, University of Glasgow for subsequent statistical analysis. The mean of the three PWA measurements was used in analysis.

Blood pressure pulse wave velocity scanning protocol

Brachial artery PWV was measured using the Sphygmocor® apparatus (PWV Medical, Sydney Australia). Whilst an electrocardiogram (ECG) was simultaneously recorded, applanation tonometry, using a hand-held piezo-resistive pressure transducer (Miller Instruments, Houston USA), was used to sequentially record, a short time apart, radial and carotid artery waveform samples of 10-second duration. The tonometer is applied to a peripheral artery site, flattening the wall of the artery. This eliminates tangential pressures and allows the pressure waveform within the artery to be recorded. The transit time between arterial sites is determined by gating the separate recordings to the R wave of the ECG. The distance between the two arterial sites was estimated using surface measurements. PWV was calculated as the distance between the two sites divided by the difference in transit time for the pressure pulse wave to reach the two sites. PWV is expressed in metres per second (m/s). This method of assessing PWV has been found to be reproducible⁵⁷⁹. The mean of three PWV measurements recorded at each visit, for the right arm, was used in the analysis.

7.2.3. Measurement of peripheral arterial tonometry reactive hyperaemia index

The first 15 patients enrolled to the study did not undergo PAT measurement as PAT measurement represented a substantial amendment to the study protocol which was implemented subsequent to the recruitment of these initial 15 patients.

Patients attended for PAT-RHI following an overnight fast and were asked to avoid caffeine, tobacco & alcohol for the preceding 12 hours. Following 15-minutes supine rest, three brachial BP measurements were taken at 1-minute intervals, using a semi-automated sphygmomanometer (Critikon DINAMAP) and the mean of these was recorded.

PAT analysis was performed in a room with controlled temperature (23 degrees Celsius), to allow acclimatisation and peripheral vasodilatation / constriction attributable to outdoor seasonal temperature variation to resolve. PAT recordings were performed using the EndoPAT 2000 device (Itamar medical, Caesarea, Israel). Pneumatic PAT probes are placed on the index finger of each hand. Following probe inflation, these probes exert a uniform pressure (70 mmHg) to the finger tip. This pressure prevents venous distension of finger volume but permit distension attributable to arterial pulsation. The probes are attached to isolated volume reservoirs which buffer any change in pressure within the probes, as occurs on a cyclic basis with the change in fingertip blood volume associated with arterial pulsation. Thus, the PAT device permits plethysmographic recording of finger arterial pulse waveform and amplitude.

Baseline pulse waveform and amplitude recordings were recorded for a period of 10 minutes, following which a brachial artery BP cuff was inflated to either 200 mmHg or 60 mmHg above systolic BP (whichever value was greater), for 5 minutes. Real time visualisation of the pulsatile wave form ensured that vascular occlusion was achieved. Following BP cuff deflation, waveform recording continued for a further 10 minutes.

The magnitude of flow-mediated fingertip hyperaemia in the occluded arm was subsequently calculated as a ratio of mean fingertip pulse amplitude during the second minute following BP cuff deflation (A) to the mean amplitude during the five minutes prior to BP cuff

inflation (B). The same ratio was calculated for the control arm (C/D), serving as control for variation associated with systemic factors. A reactive hyperaemia index (RHI) was calculated as $(A/B) / (C/D)$. Calculation of RHI was performed in an automated, operator-independent fashion, using EndoPAT proprietary software. The selection of timing of recordings for RHI calculation is based on these holding the strongest correlation with endothelial function determined at coronary angiography⁶²³.

7.2.4. Measurement of circulating markers of endothelial function

Blood was sampled at baseline and 6 month follow up for measurement of circulating levels of Von Willebrand factor (vWF), soluble intercellular adhesion molecule 1 (s-ICAM-1), e-selectin & s-thrombomodulin. Highly sensitive ELISA techniques, utilising the quantitative sandwich enzyme immunoassay technique, were used, as previously described⁴¹⁴. s-ICAM-1, e-selectin and s-thrombomodulin assays were performed using Quantizing ELISA kits (R&D systems). vWF assays were performed using the Technozym ELISA kit (Pathway Diagnostics). e-Selectin and s-ICAM-1 levels were measured from serum, vWF and thrombomodulin were measured from citrated plasma. Both sample types were stored at -80 degrees Celsius. Laboratory analysis was performed by DH.

7.2.5. Protocol amendments

Ethical approval was given following peer review of the study protocol (protocol version 1) by the West Glasgow Ethics Committee 2 on 25/8/2008. The study protocol was approved by local NHS R&D management and by the MHRA (UK). Two substantial amendments were made to the original study protocol, dated 19/1/2010 (protocol version 2) and 5/8/2010 (protocol version 3). These amendments were approved by the West Glasgow Ethics Committee 2, local NHS R&D management and the MHRA.

In brief, the first substantial amendment made provision for BP-PWA endpoint data to also be collected at the 1 month study visit. The amendment also allowed for the additional non-invasive measurement of endothelial function in study patients at baseline, 1 month and 6

month study visits. This was performed with measurement of digital reactive hyperaemia index following FMD in the upper limb. This amendment was applicable to participants 016 through 080, inclusive, enrolled to the study.

The second substantial amendment allowed for the extension of study treatment from 12 to 24 months, in patients who provided informed consent, with additional measurement of CIMT at 24 month follow up. These 24 month data would be available for inclusion in any subsequent meta-analysis.

Statistical analysis

A statistical analysis plan was conceived then revised following discussion with consultant statisticians (HM & AM), based at the Robertson Centre for Biostatistics at the University of Glasgow. Dummy generated data was used to populate statistics reports which were reviewed by investigators and statisticians prior to analysis of the trial sample data with unlocked treatment allocation code, which was performed by the Robertson Centre for Biostatistics (HM & AM), University of Glasgow.

All analyses were performed on an intention to treat basis, based on complete data available at baseline and final follow up for each endpoint. Study comparisons on continuous measurements were performed with analysis of covariance (ANCOVA) adjusted for baseline value and treatment allocation and results are expressed as the change in allopurinol group value minus the change in placebo group value. Sensitivity analysis was performed for all efficacy endpoints to assess sensitivity to extreme values. Comparisons on categorical variables were performed with difference in proportions. A p value of < 0.05 was considered statistically significant.

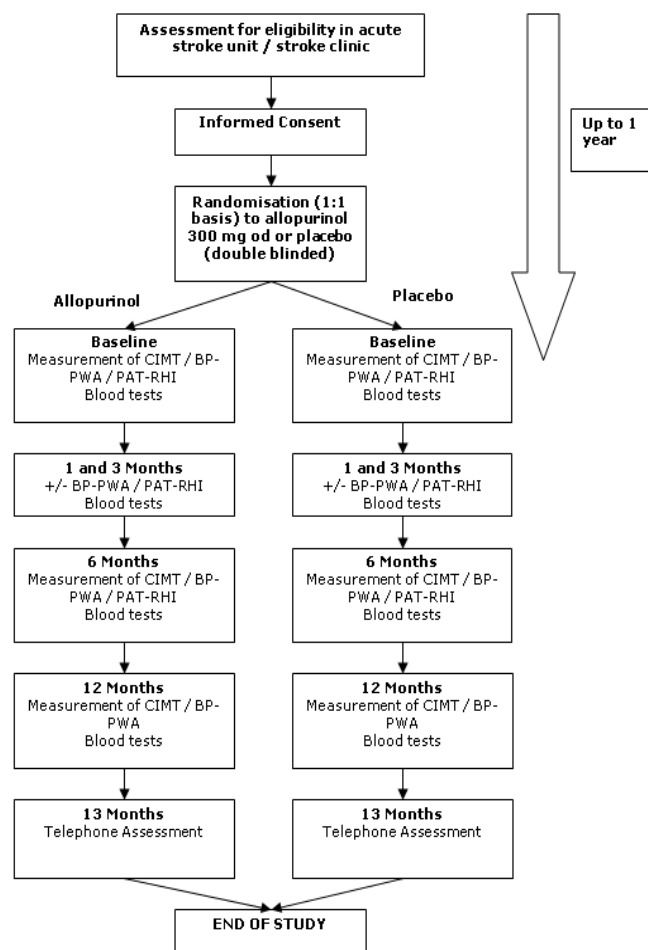
A priori primary study endpoints included the difference in mean maximum CIMT progression, mean common CIMT progression, change in central BP, AI, AI standardised for HR of 75 bpm (AI@HR75), carotid-radial PWV and brachial BP, between treatment groups over a one year period. The safety analysis included the number of serious adverse events attributable to therapy and the number of all serious adverse events and all adverse events.

Secondary endpoints included; the difference in change in SUA level between treatment groups, at 1, 3, 6 & 12 months; the difference in mean maximum CIMT progression, mean common CIMT progression and change in circulating markers of endothelial activation, between treatment groups, at 6 months; the difference in change in central BP, AI, AI (standardised for HR of 75 bpm), carotid-radial PWV, brachial BP and PAT-RHI, between treatment groups, at 1 & 6 months.

Secondary post hoc analyses were planned to correlate CIMT and central BP measurements at baseline with SUA and to correlate change in CIMT and central BP with change in SUA, to evaluate whether these parameters appeared related to serum UA level.

An outline of study procedures is included in figure 7-1.

Figure 7-1: Summary of Chapter 7 study design



7.3. Study One Results

80 participants were recruited between September 2009 and November 2010 (mean age (SD) 67.8 (9.4) years). 12 month follow up visits completed on 8/11/2011. 25 patients consented to proceed to 24 month follow up. 24 month follow up visits completed on 13/11/2012. Statistical analysis for the 12 month original Pilot phase was completed in June 2013.

Baseline demographic characteristics and study endpoint parameter values at baseline, , are shown, respectively, in tables 7-2 and 7-3. Endpoint parameter values at baseline, based on the full randomised population and for each subgroup of patients for whom interval data was available for comparison, are shown in appendix C (table C-1). Baseline values for those patients with data available for interval analysis were comparable to those for the randomised population. Treatment groups were generally well balanced although the placebo group contained a greater proportion of participants with index event of stroke (rather than TIA) and diabetes whilst fewer had documented hypertension, AF or treatment with ACE-I / ARB agents.

Table 7-2: Allopurinol CIMT study population baseline characteristics

Variable	All Participants (n=80)	Allopurinol (n=40)	Placebo (n=40)	P-value
Age (years)	67.9 (9.50)	66.9 (8.72)	68.8 (10.25)	0.38
Male Sex	46 (57.5%)	60%	55%	0.65
Smoker	15 (18.75%)	20%	17.5%	0.77
BMI	26.9 (5.48)	26.1 (4.52)	27.7 (6.24)	0.21
Brachial systolic BP (mmHg)	135.2 (19.24)	135.3 (20.30)	135.2 (18.39)	0.97
Brachial diastolic BP (mmHg)	74.9 (8.27)	76.1 (7.81)	73.7 (8.64)	0.19
Serum Cholesterol (mmol/l)	4.01 (0.81)	4.13 (0.92)	3.90 (0.66)	0.22
Serum Glucose (mmol/l)	5.85 (1.59)	5.53 (1.24)	6.16 (1.84)	0.08

Serum Creatinine (μmol/l)	77.59 (15.08)	78.8 (14.95)	76.4 (15.31)	0.48
Serum Uric Acid (mmol/l)	0.32 (0.09)	0.32 (0.09)	0.31 (0.08)	0.56
Time from ictus to randomisation (days)	66 (20-104)	71.5 (25.5 – 110.0)	56.0 (15.5-101.5)	0.39 ¹
Qualifying Event for Trial				
Stroke	58.75%	50%	67.5%	0.11
TIA	41.25%	50%	32.5%	
OCSF				0.46 ²
TACS	5%	7.5%	2.5%	
LACS	50%	42.5%	57.5%	
PACS	32.5%	37.5%	27.5%	
POCS	11.25%	12.5%	10%	
Amaurosis Fugax	1.25%	0	2.5%	
IHD	10.00%	7.50%	12.50%	0.71 ²
Diabetes	13.75%	7.50%	20.00%	0.19 ²
Previous Stroke or TIA	17.5%	15%	20%	0.56
Hypertension	42.50%	50.00%	35.00%	0.17
AF	10.00%	15.00%	5.00%	0.26 ²
Anti-platelet Therapy	92.5%	95%	90%	0.39
ACE Inhibitor or ARB Therapy	48.75%	60%	37.5%	0.044
Diuretic Therapy	31.25%	22.5%	40%	0.09
CCB	16.5%	20.0%	12.5%	0.55 ²
Beta-Blocker Therapy	28.75%	12.5%	45%	0.003 ²
Lipid Lowering Therapy	92.5%	95%	90%	0.39
<p>Data are expressed as mean (SD) for continuous variables and % for categorical variables, with the exception of time from ictus to randomisation & the circulating endothelial function parameters, where median (interquartile range is given). For continuous variables, p-values are from two-sample t-test, except when a variable did not approximate to the Normal distribution when the Mann-Whitney test ¹ was used. For categorical variables p-values are from Chi-squared test, except for this denoted by ² which are from Fisher's exact test. A p value of < 0.05 was considered statistically significant. BMI, body mass index; TIA, transient ischemic attack; OCSF, Oxford Clinical Stroke Project; AF, atrial fibrillation; IHD, ischaemic heart disease; TACS, total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar stroke; POCS, posterior circulation stroke; mRS, modified Rankin scale; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.</p>				

Table 7-3: Allopurinol study population endpoint parameter baseline values

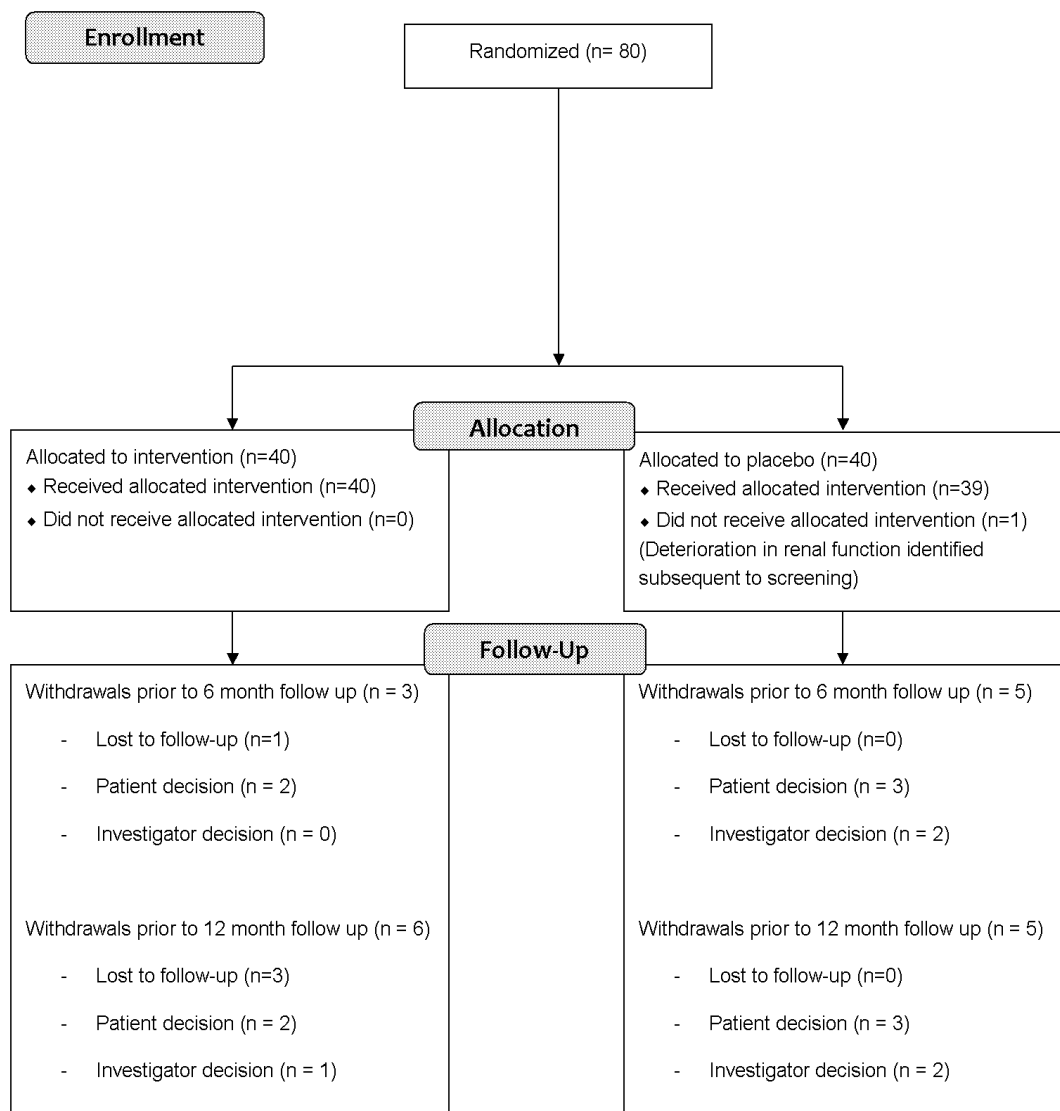
Study sample (n, Allopurinol / Placebo)	All Participants	Allopurinol	Placebo	P-value
Uric acid, mmol / litre (37/40)	0.32 (0.09)	0.32 (0.09)	0.31 (0.08)	0.56
Mean maximum CIMT, mm (39/40)	1.18 (0.31)	1.20 (0.32)	1.17 (0.30)	0.70
Mean common (far wall CIMT), mm (39/40)	0.83 (0.15)	0.83 (0.16)	0.82 (0.15)	0.83
Mean aortic SBP, mmHg (40/39)	111.7 (14.9)	112.3 (15.8)	111.0 (14.0)	0.71
Mean aortic DBP, mmHg (40/39)	90.0 (10.1)	91.0 (10.6)	88.9 (9.6)	0.37
Mean aortic Tr, ms (40/38)	135.8 (10.00)	136.4 (9.43)	135.2 (10.67)	0.62
Mean augmentation, mmHg (40/39)	17.9 (7.9)	17.3 (7.5)	18.5 (8.3)	0.50
AI (P2/P1), % (40/39)	153.7 (17.8)	152.0 (15.03)	155.4 (20.3)	0.40
AI (AG/PP), % (40/39)	34.2 (7.7)	33.9 (7.0)	34.6 (8.5)	0.69
AI@HR75, % (40/39)	28.8 (7.9)	28.4 (8.1)	29.3 (7.9)	0.60
Mean end systolic pressure, mmHg (40/39)	114.4 (15.4)	114.5 (16.0)	114.3 (14.9)	0.96
PWV (brachial), m/s (33/35)	8.48 (0.98)	8.55 (1.01)	8.41 (0.96)	0.57
Brachial SBP, mmHg (40/40)	135.2 (19.2)	135.3 (20.3)	135.2 (18.4)	0.97

Brachial DBP, mmHg (40/40)	74.9 (8.3)	76.1 (7.8)	73.7 (8.6)	0.19
PAT-RHI (32/30)	2.4 (0.79)	2.5 (0.91)	2.2 (0.61)	0.15
ICAM-1 (37/35)	263.8 (89.0)	238.4 (71.3)	290.7 (98.5)	0.012
e-Selectin (37/35)	36.2 (15.1)	32.6 (13.8)	40.0 (15.8)	0.038
vWF (37/35)	1.29 (0.42)	1.35 (0.39)	1.23 (0.44)	0.26
Thrombomodulin (37/35)	3299 (838)	3283 (860)	3315 (826)	0.87
Data are expressed as mean (standard deviation). P-values are from two-sample t- test. CIMT, carotid intima media thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; PWV, pulse wave velocity; PAT-RHI, peripheral artery tonometry reactive hyperaemia index; ICAM-1, intercellular adhesion molecule-1; vWF, von Willebrand Factor.				

Follow up

In total, 72 participants completed 6 month follow up and 69 completed 12 month follow up. Vital status was ascertained for all participants except one, who left the country and was lost to follow up. Of the remaining 10 patients with incomplete 12 month follow up, 3 withdrew following adverse events respectively of brain tumour, pancreatic carcinoma and depressive psychosis; 5 chose to withdraw and 2 did not attend for the final visit despite repeated attempts to schedule appointments. A CONSORT diagram detailing recruitment and withdrawals is shown in figure 7-2.

Figure 7-2: Patient follow up at 6 & 12 months - CONSORT 2010 Flow Diagram



Study treatment concordance

91% of patients took at least 80% of their prescribed study medication doses based on returned pill counts and withdrawals as detailed above. One patient (who subsequently withdrew) received no study medication, as renal function had deteriorated subsequent to initial screening by the time of randomisation. They were included in the intention to treat analysis.

Safety Analysis

2 patients had developed neutropenia at 1 month follow up (one attributed to intercurrent viral infection, the other potentially to study medication). 1 patient developed an urticarial rash within one week (likely due to concomitant nicotine replacement patches). Study medication was immediately discontinued without rechallenge in all 3 patients. All 3 patients were in the placebo group on unblinding of treatment allocation on study completion. No other study treatment related serious adverse events or adverse reactions were reported.

Serum Uric Acid

Serum UA values at baseline are detailed in table 7-3. Between September 2009 and November 2011, the local NHS laboratory uric acid assay showed an average bias of +3.3% to the target values assigned by UKNEQAS (based on the mean results for all labs using the endpoint uricase method). This bias was largely due to the local method, which used Abbott reagents.

Change in SUA at 1, 3, 6 & 12 months, together with intergroup comparisons of change from baseline, are detailed in table 7-4. Serum UA level fell significantly with allopurinol compared with placebo at each follow up time point. Repeated statistical analysis, with adjustment for baseline treatment with ACE-I / ARB (which differed significantly between treatment groups), did not affect the observed results (table 7-5). Figure 7-3 illustrates change in SUA from baseline during follow up in each group.

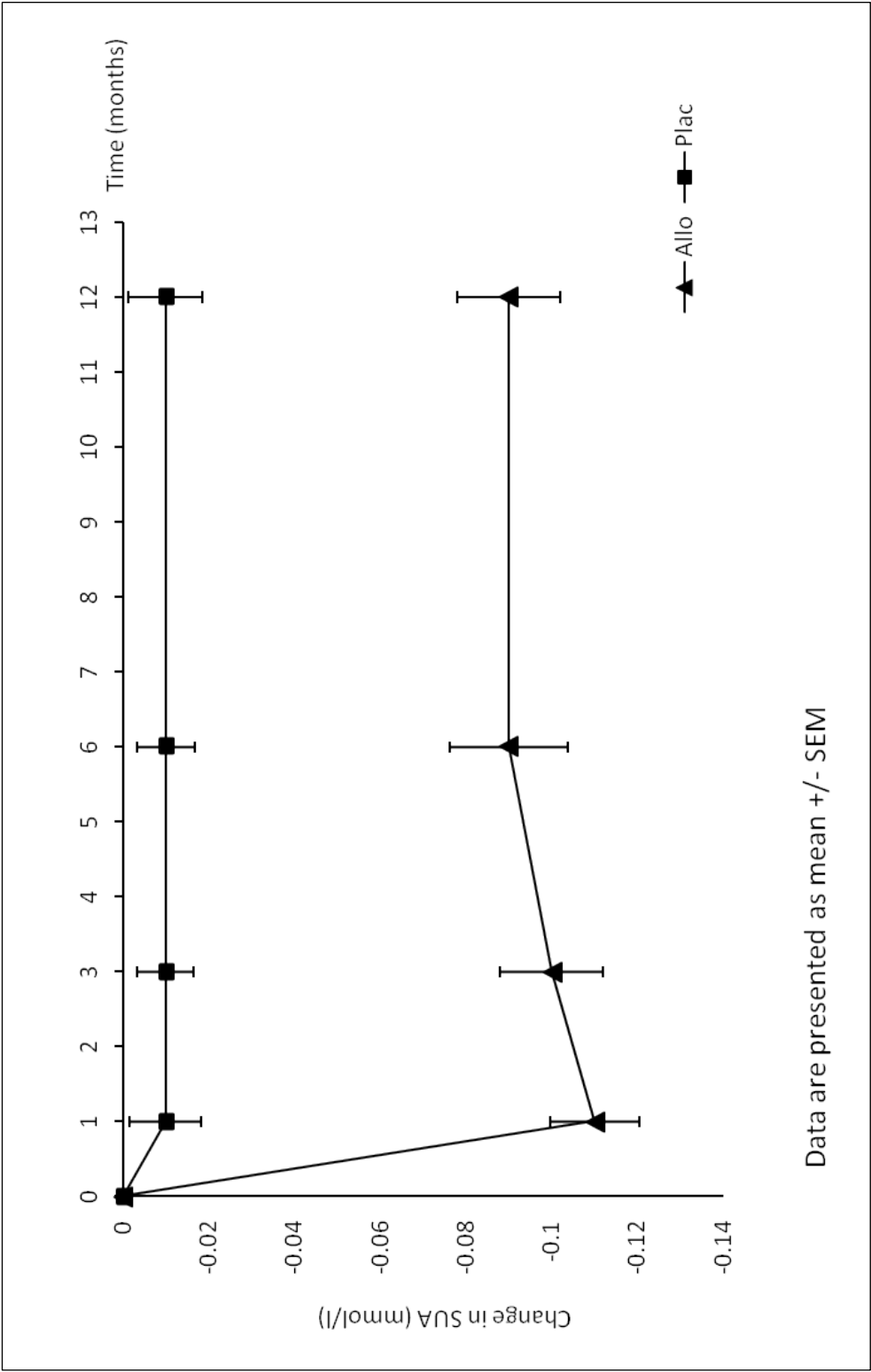
Table 7-4: Change in serum uric acid at 1, 3, 6 & 12 month follow up

Follow up month (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference (95% CI)	P value
1 month (33 / 36)	-0.11 (0.06)	-0.01 (0.05)	-0.100 (-0.122 , -0.077)	< 0.0001
3 months (34 / 37)	-0.10 (0.07)	-0.01 (0.04)	-0.086 (-0.111 , -0.061)	< 0.0001
6 months (34 / 35)	-0.09 (0.08)	-0.01 (0.04)	-0.070 (-0.096, -0.044)	< 0.0001
12 months (34 / 34)	-0.09 (0.07)	-0.01 (0.05)	-0.076 (-0.104, -0.048)	< 0.0001
Data are mmol/litre, presented as mean (SD), unless otherwise stated.				

**Table 7-5: Change in serum uric acid at 6 & 12 month follow up
(adjusted for baseline treatment with ACE-I / ARB)**

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
1 month (33 / 36)	-0.100 (-0.122 , -0.077)	-0.101 (-0.124 , -0.077)	< 0.0001
3 months (34 / 37)	-0.086 (-0.111 , -0.061)	-0.086 (-0.111 , -0.061)	< 0.0001
6 months (34 / 35)	-0.070 (-0.096, -0.044)	-0.070 (-0.097, -0.043)	< 0.0001
12 months (34 / 34)	-0.076 (-0.104, -0.048)	-0.077 (-0.106, -0.048)	< 0.0001
Data are mmol/litre, presented as mean (95% confidence interval).			

Figure 7-3: Change in serum uric acid between baseline and subsequent follow-up



7.3.1. CIMT analysis

Baseline mean maximum CIMT and mean common CIMT values appeared balanced between treatment groups and are detailed in table 7-3. Carotid CIMT measurements meeting the dimension based criteria for presence of carotid plaque, of at least one angle measurement at any of the three carotid segments, indicated that carotid plaque was present in 56 patients (70.9%). No difference was observed in the proportion of patients with presence of carotid plaque between treatment groups at baseline: allopurinol, n = 30 (76.92%); placebo, n = 26 (65.00%), $p = 0.33$ (for difference in 2 proportions). Figures C-1 and C-2 (appendix C) illustrate, respectively, the spread of mean maximum and mean common (far wall) CIMT measurements at baseline.

67 patients had CIMT data recorded at both baseline and 6 month visits for comparison. No significant difference was observed between the treatment groups, for either mean maximum or mean common CIMT progression, at 6 month follow up. A sensitivity analysis, with censoring of observed extreme values, was performed for mean maximum CIMT at 6 months. Similar results were obtained in this analysis. Change in mean maximum and mean common CIMT values between baseline and 6 months within each treatment group, together with comparison of intergroup changes from baseline, are detailed in table 7-6.

Table 7-6: CIMT parameter progression at 6 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference (95% CI)	P value
Mean maximum (35 / 32)	0.04 (0.20)	0.05 (0.21)	-0.005 (-0.101, 0.091)	0.91
Mean maximum (sensitivity analysis) (34 / 31)	0.02 (0.16)	0.02 (0.14)	0.002 (-0.070, 0.074)	0.96
Mean common (far wall) (35 / 32)	-0.00 (0.07)	0.00 (0.12)	-0.006 (-0.050, 0.038)	0.79
Data are mm, presented as mean (SD), unless otherwise stated.				

65 patients had IMT data recorded at both baseline and 12 month visits for comparison. A CONSORT diagram, detailing explanations for patient unavailability for this analysis is shown in appendix C (figure C-3).

Change in mean maximum and mean common CIMT values between baseline and 12 months within each treatment group, together with comparison of intergroup changes from baseline, are detailed in table 7-7. No significant difference was observed between treatment groups in mean maximum CIMT progression at 12 months (-0.060mm; 95% CI, -0.146, 0.027mm, $p = 0.17$). Mean common CIMT progression was significantly reduced in the Allopurinol group compared with the Placebo group at 12 month follow up (-0.097mm, 95% CI; -0.175, -0.019mm, $p=0.015$).

A sensitivity analysis, with censoring of observed extreme values, was performed for mean common CIMT at 12 months. The treatment effect was preserved in this analysis (-0.057mm, 95% CI; -0.108, -0.006mm, $p = 0.029$). Change in CIMT parameters from baseline during follow up in each group are shown in figures 7-4, 7-5 & 7-6.

Table 7-7: CIMT parameter progression at 12 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference (95% CI)	P value
Mean maximum (31 / 34)	0.01 (0.15)	0.07 (0.19)	-0.060 (-0.146, 0.027)	0.17
Mean common (far wall) (31 / 34)	-0.02 (0.09)	0.08 (0.20)	-0.097 (-0.175, -0.019)	0.015
Mean common (far wall), (sensitivity analysis) (31 / 32)	-0.02 (0.09)	0.04 (0.11)	-0.057 (-0.108, -0.006)	0.029
Data are mm, presented as mean (SD), unless otherwise stated.				

Figure 7-4: Change in mean maximum CIMT (mm) between baseline and subsequent follow-up

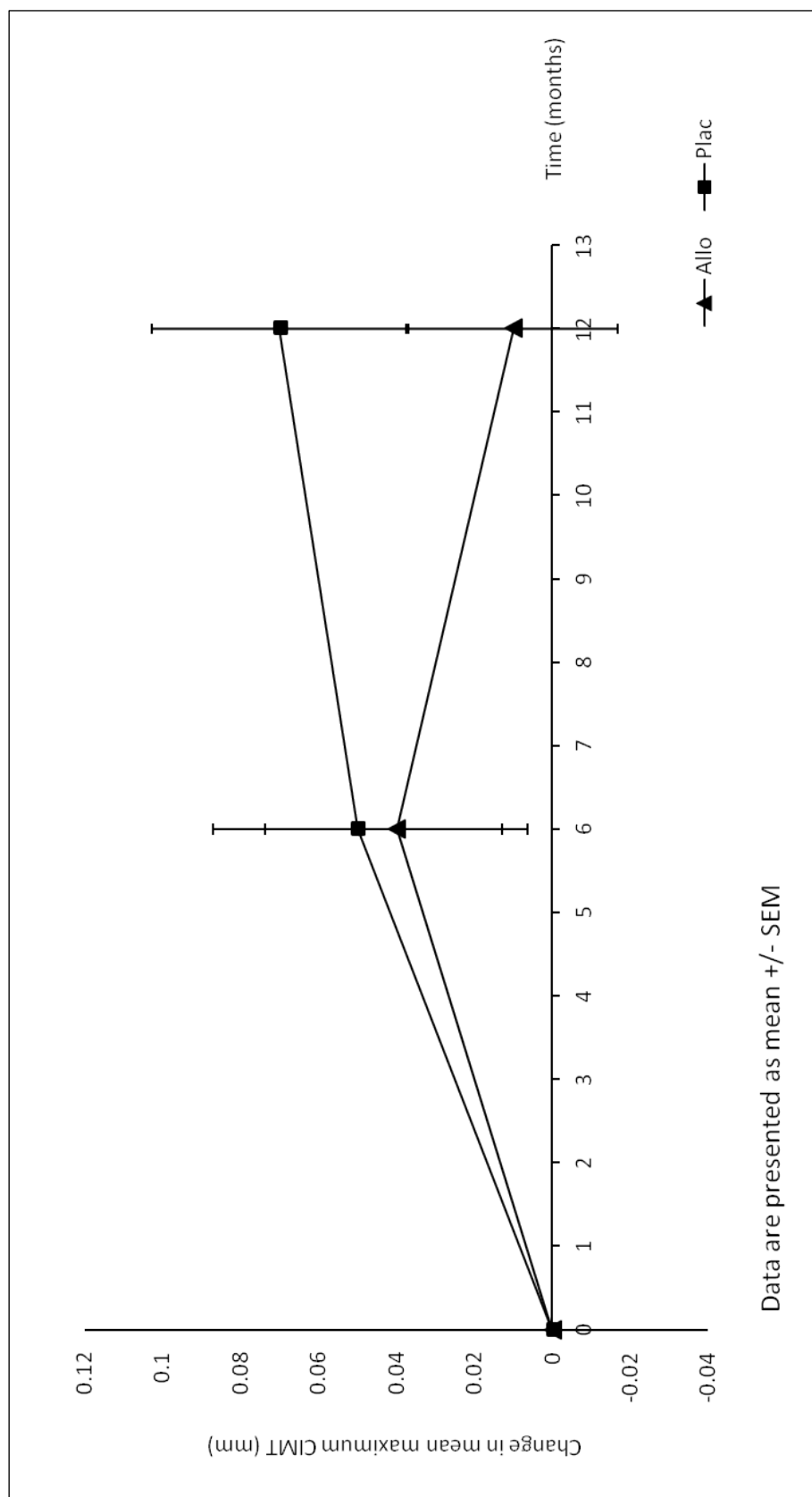


Figure 7-5: Change in mean common (far wall) CIMT (mm) between baseline and subsequent follow-up

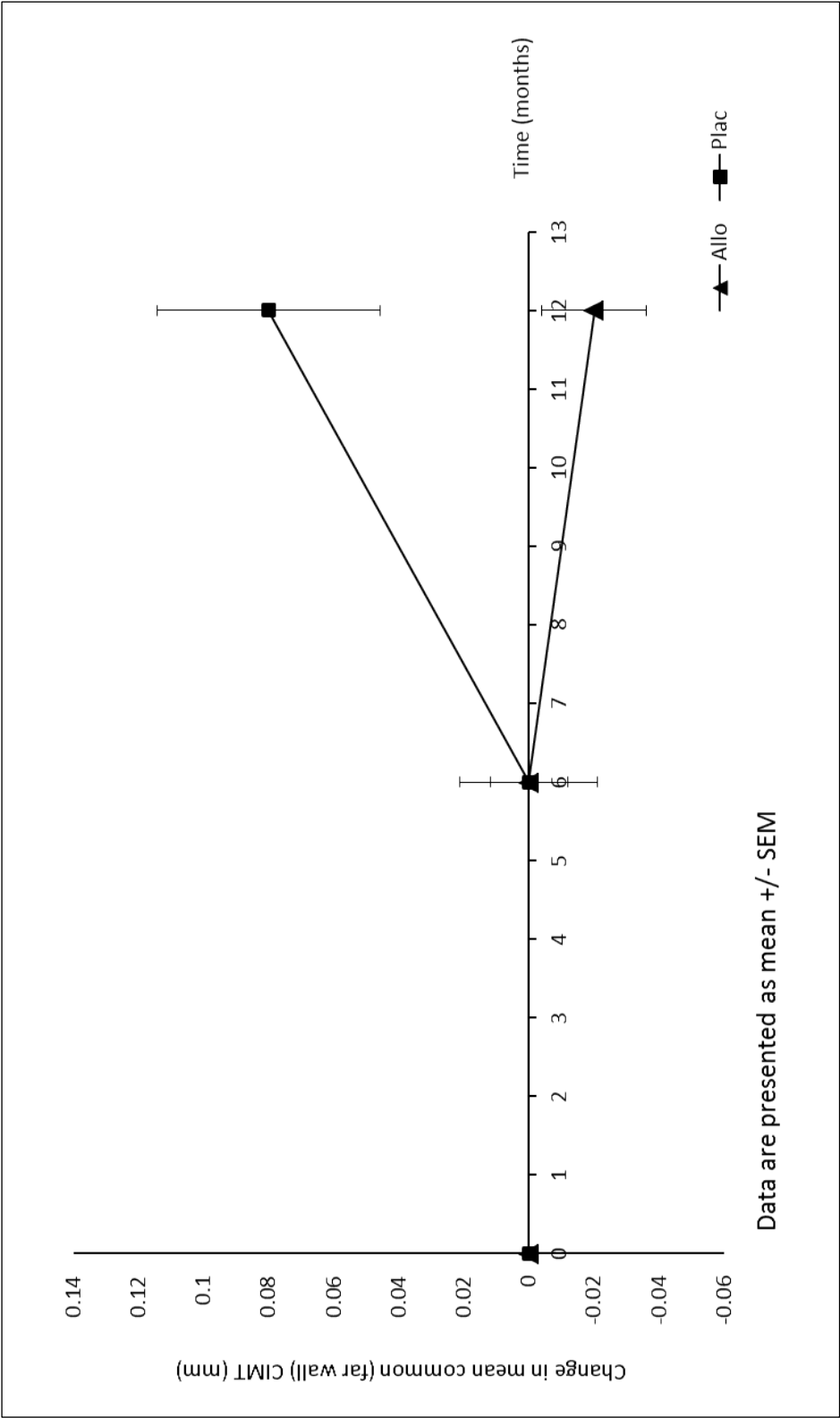
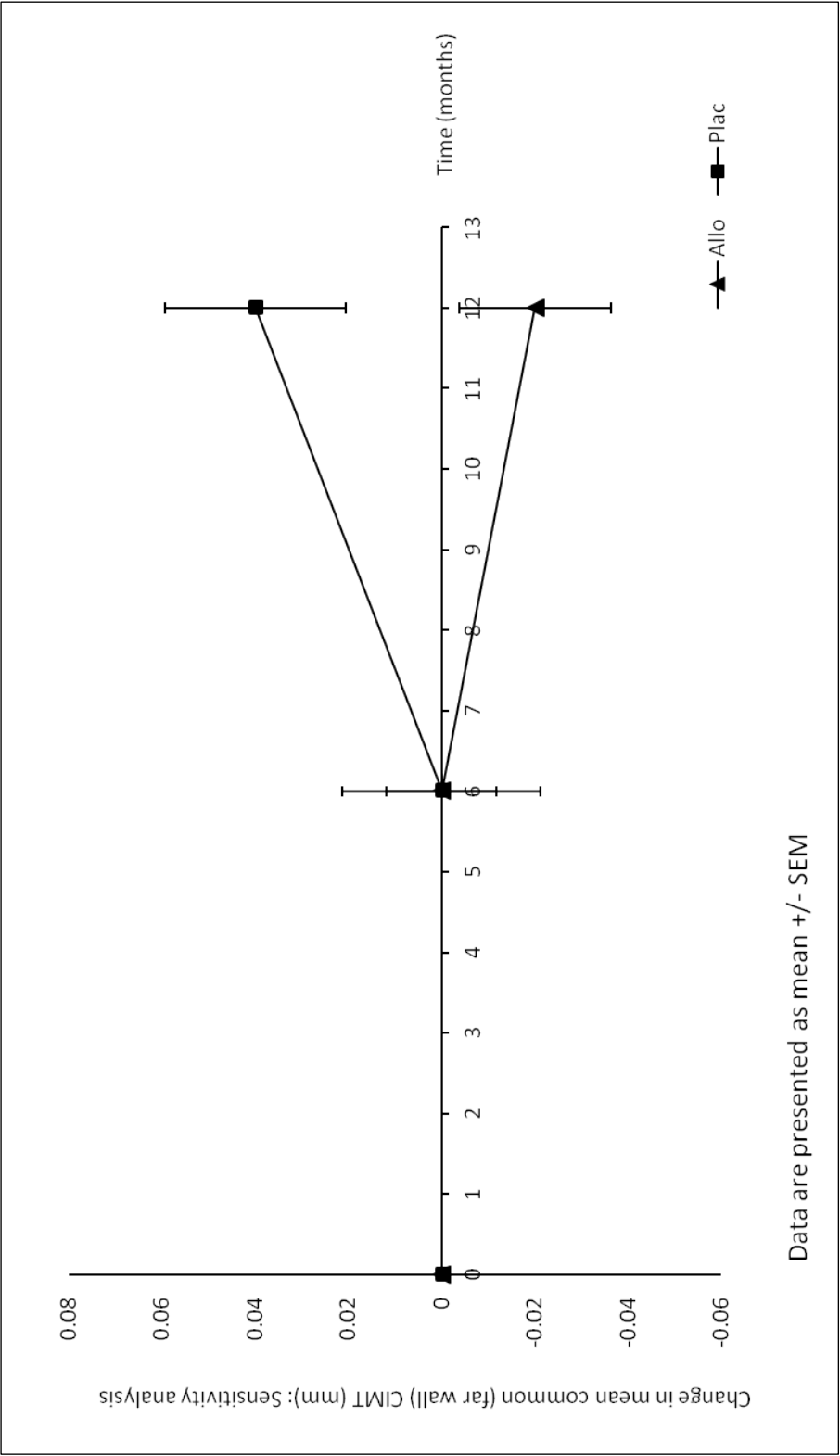


Figure 7-6: Change in mean common (far wall) ICMT (mm) between baseline and subsequent follow-up
(sensitivity analysis)



IMT Reproducibility

There was satisfactory intra-observer agreement for CIMT image analysis for both mean maximum and mean common CIMT. The within observer intra-class correlation (ICC) for mean maximum CIMT was 0.91 (95% CI; 0.79, 0.96), based on repeated analysis of 20 participants mean maximum CIMT at baseline. A Bland-Altman plot (figure 7-7) for the intra-observer variability did not demonstrate any trend for variability to be dependent on the underlying mean value for mean maximum carotid IMT. The within observer ICC for mean common (far wall) CIMT was 0.87 (95% CI; 0.77, 0.93), based on repeated analysis of 40 participants mean common CIMT at baseline. A Bland-Altman plot (figure 7-8) for the intra-observer variability did not demonstrate any trend for variability to be dependent on the underlying mean value for mean common (far wall) carotid IMT.

Figure 7-7: Intra –observer agreement for mean maximum CIMT analysis

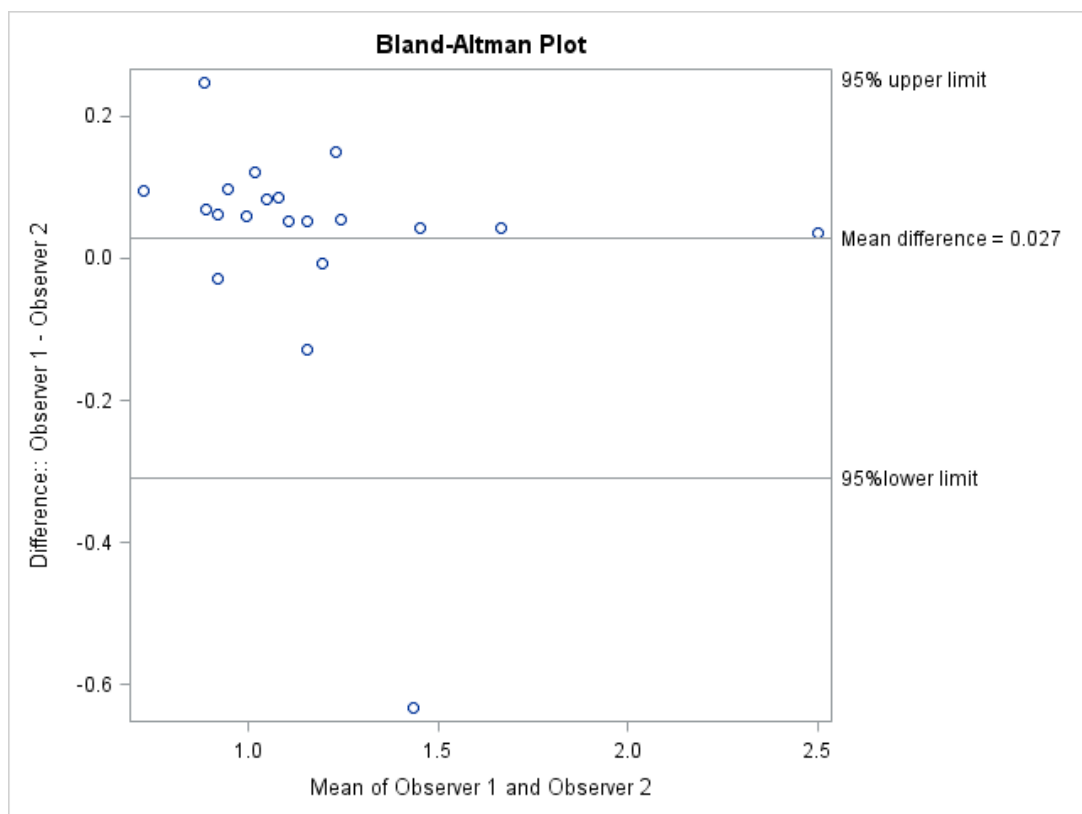
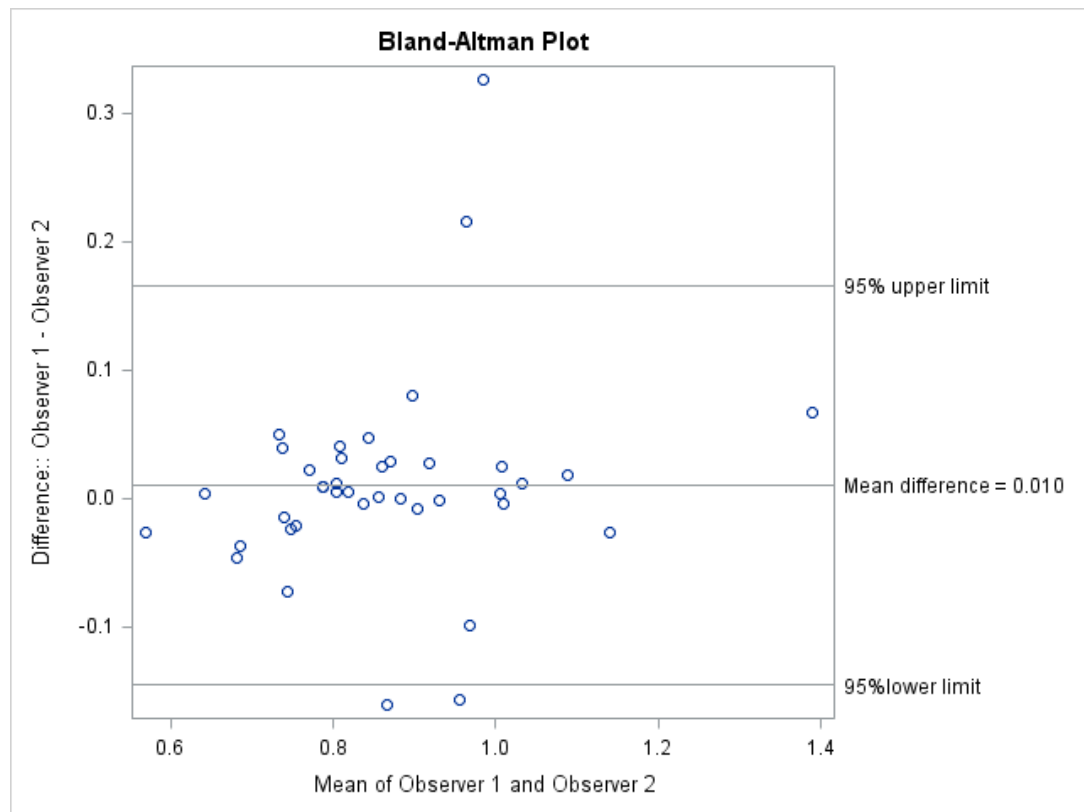


Figure 7-8: Intra –observer agreement for mean common (far wall) CIMT analysis



Post hoc analyses

The difference in the proportion of patients treated with ACE-I or ARB between treatment groups at baseline was significant. This potentially favoured the observation of a treatment effect in favour of Allopurinol. We therefore repeated statistical analysis with adjustment for baseline ACE-I or ARB use. The adjusted intergroup differences for mean maximum CIMT and mean common CIMT progression at 6 and 12 months are detailed in table 7-8. The results appeared unaffected by this adjustment.

On identifying a statistically significant difference in 12 month mean common CIMT progression between treatment groups, we performed correlation between baseline serum UA and baseline mean common CIMT and between change in serum UA at 6 and 12 months and change in mean common CIMT at 6 & 12 months. The correlation coefficient and accompanying p value for each correlation are detailed in table 7-9. A non-significant trend toward a linear correlation between baseline mean maximum CIMT and baseline SUA was observed. No correlation between the observed change in SUA and change in mean

common CIMT at either 6 or 12 months was apparent. Repeated statistical analysis, including adjustment for the observed change in SUA at 12 months, did not affect the difference in mean common CIMT progression observed between treatment groups (-0.102mm, 95% CI; -0.195, -0.008mm, p=0.033).

Table 7-8: CIMT parameter progression at 12 month follow up, adjusted for baseline treatment with ACE-I / ARB at baseline

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
Mean maximum CIMT (31 / 34)	-0.060 (-0.146, 0.027)	-0.061 (-0.149, 0.028)	0.18
Mean common CIMT (31 / 34)	-0.097 (-0.175, -0.019)	-0.097 (-0.176, -0.017)	0.019
Mean common CIMT (sensitivity analysis) (31 / 32)	-0.057 (-0.108, -0.006)	-0.062 (-0.113, -0.011)	0.018
Data are mm, presented as mean (95% confidence interval).			

Table 7-9: CIMT correlations with Uric Acid

	Allopurinol	Placebo	All
Baseline	0.161 (0.34)	0.245 (0.127)	0.181 (0.11)
Change at 6 months	0.094 (0.60)	0.197 (0.28)	0.169 (0.18)
Change at 12 months	0.051 (0.79)	0.020 (0.91)	0.150 (0.24)
Data are presented as correlation coefficient (p value)			

7.3.2. BP-PWA analysis

Baseline BP-PWA values are detailed in table 7-3. 60 patients had PWA data recorded at both baseline and 1 month visits for comparison. Incomplete BP-PWA data, due to partial technical inadequacy of PWA signal acquisition, meant determination of mean peripheral T2, mean peripheral AI was based on 57 patients. Similarly, determination of mean aortic Tr was based on 58 patients.

Change in BP-PWA parameter values between baseline and 1 month within each treatment group, together with comparison of intergroup differences from baseline, are detailed in table 7-10. Adjustment for baseline treatment with ACE-I / ARB did not affect the observed results (table 7-11).

Table 7-10: BP-PWA parameter change at 1 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol mean (SD)	Placebo mean (SD)	Mean intergroup difference, (95% CI)	P value
Mean aortic SBP (32/28)	-3.8 (10.1)	-3.1 (9.8)	-0.3 (-5.1 , 4.6)	0.91
Mean aortic DBP (32/28)	-3.3 (8.1)	-3.1 (8.5)	0.443 (-3.6 , 4.5)	0.83
Mean aortic Tr, ms (32/26)	0.39 (6.17)	-0.96 (13.73)	2.52 (-1.670 , 6.73)	0.24
Mean augmentation (32/28)	-2.0 (4.8)	-0.1 (6.9)	-2.2 (-5.0 , 0.6)	0.13
AI (P2/P1), % (32/28)	-6.3 (10.9)	0.5 (16.5)	-7.3 (-14.2 , -0.5)	0.036
AI (AG/PP), % (32/28)	-3.0 (5.6)	0.1 (6.6)	-3.1 (-6.2 , -0.0)	0.048
AI@HR75, % (32/28)	-2.1 (5.8)	-0.1 (5.6)	-2.3 (-5.1 , 0.6)	0.12
Mean ESP, mmHg (32/28)	-4.9 (11.4)	-3.5 (10.2)	-1.4 (-6.6 , 3.9)	0.60
Data are expressed as mean (standard deviation), unless otherwise stated. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure				

**Table 7-11: BP-PWA parameter change at 1 month follow up,
adjusted for treatment with ACE-I / ARB at baseline**

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
Mean aortic SBP, mmHg	-0.3 (-5.1 , 4.6)	0.2 (-4.7 , 5.2)	0.93
Mean aortic DBP, mmHg	0.443 (-3.6 , 4.5)	0.6 (-3.5 , 4.7)	0.78
Mean aortic Tr, ms	2.52 (-1.670 , 6.73)	1.90 (-2.43 , 6.23)	0.38
Mean augmentation	-2.2 (-5.0 , 0.6)	-1.5 (-4.3 , 1.2)	0.27
AI (P2/P1), %	-7.3 (-14.2 , -0.5)	-6.3 (-13.2 , 0.6)	0.07
AI (AG/PP), %	-3.1 (-6.2 , -0.0)	-2.7 (-5.8 , 0.5)	0.09
AI@HR75, %	-2.3 (-5.1 , 0.6)	-1.9 (-4.8 , 1.0)	0.20
Mean ESP, mmHg	-1.4 (-6.6, 3.9)	-0.7 (-6.1 , 4.6)	0.78
Data are expressed as mean (95% confidence interval). SBP, systolic blood pressure; DBP, diastolic blood pressure; Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure			

68 patients had PWA data recorded at both baseline and 6 month visits for comparison. One patient in the placebo group had incomplete BP-PWA data, such that determination of mean peripheral T2, mean peripheral AI and mean aortic Tr was based on 67 patients.

Change in BP-PWA parameter values between baseline and 6 months within each treatment group, together with comparison of intergroup differences from baseline, are detailed in table 7-12. Adjustment for baseline treatment with ACE-I / ARB did not affect the observed results (table 7-13).

Table 7-12: BP-PWA parameter change at 6 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
Mean aortic SBP, (35 / 33)	-2.6 (13.5)	0.3 (12.8)	-2.2 (-8.5, 4.2)	0.50
Mean aortic DBP (35 / 33)	-2.4 (10.0)	-0.6 (10.3)	-1.1 (-5.9, 3.8)	0.67
Mean aortic Tr, ms (35 / 32)	0.60 (8.22)	-0.27 (11.32)	1.27 (-2.28, 4.83)	0.48
Mean augmentation (35 / 33)	-1.4 (6.5)	0.6 (6.7)	-1.9 (-4.8, 1.0)	0.20
AI (P2/P1), % (35 / 33)	-3.3 (14.4)	-2.8 (26.3)	-0.9 (-11.0, 9.1)	0.85
AI (AG/PP), % (35/33)	-2.02 (6.5)	3.4 (21.2)	-5.4 (-12.3, 1.5)	0.13
AI@HR75, % (35 / 33)	-1.2 (5.8)	-0.2 (6.6)	-1.1 (-4.0 , 1.8)	0.45
Mean ESP, mmHg (35 / 33)	-2.8 (13.9)	-0.8 (14.1)	-1.4 (-8.0, 5.2)	0.68
Data are expressed as mean (standard deviation), unless otherwise stated. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure				

**Table 7-13: BP-PWA parameter change at 6 month follow up,
adjusted for treatment with ACE-I / ARB at baseline**

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
Mean aortic SBP, mmHg	-2.2 (-8.5, 4.2)	-2.2 (-8.5, 4.2)	0.50
Mean aortic DBP, mmHg	-1.1 (-5.9, 3.8)	-1.0 (-6.0, 4.0)	0.68
Mean aortic Tr, ms	1.27 (-2.28, 4.83)	0.78 (-2.82, 4.38)	0.67
Mean augmentation	-1.9 (-4.8, 1.0)	-1.8 (-4.8,1.2)	0.23
AI (P2/P1), %	-0.9 (-11.0, 9.1)	0.7 (-9.3, 10.7)	0.89
AI (AG/PP), %	-5.4 (-12.3, 1.5)	-5.4 (-12.6, 1.7)	0.13
AI@HR75, %	-1.1 (-4.0 , 1.8)	-0.8 (-3.7, 2.2)	0.61
Mean ESP, mmHg	-1.4 (-8.0, 5.2)	-1.3 (-8.1, 5.4)	0.69
Data are expressed as mean (95% confidence interval). SBP, systolic blood pressure; DBP, diastolic blood pressure; Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure			

57 patients had PWA data recorded at both baseline and 12 month visits for comparison. A CONSORT diagram, detailing explanations for patient unavailability for this analysis is shown in appendix C (figure C-4). One patient in the placebo group had incomplete BP-PWA data, such that determination of mean peripheral T2, mean peripheral AI, mean aortic AI@HR75 and mean aortic Tr was based on 56 patients.

Change in BP-PWA parameter values between baseline and 12 months within each treatment group, together with comparison of intergroup differences from baseline, are detailed in table 7-14. Mean aortic SBP and augmentation index were each significantly reduced in the Allopurinol group compared with the Placebo group, at 12 month follow up. AI, corrected for heart rate of 75 bpm, was non-significantly reduced in the Allopurinol group. Adjustment for baseline treatment with ACE-I / ARB did not affect the observed results (table 7-15). Changes in aortic mean systolic BP and in AI, from baseline during follow up, for each treatment group, are illustrated in figures 7-9 and 7-10.

Table 7-14: BP-PWA parameter change at 12 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
Mean aortic SBP (30 / 27)	-4.2 (13.3)	3.2 (11.0)	-6.6 (-13.0 , -0.3)	0.042
Mean aortic DBP (30 / 27)	-3.5 (10.0)	0.4 (8.1)	-2.8 (-7.4 , 1.8)	0.23
Mean aortic Tr, ms (30 / 26)	0.42 (7.33)	-0.33 (5.56)	1.43 (-1.92 , 4.77)	0.40
Mean augmentation (30 / 27)	-1.6 (7.7)	2.1 (5.8)	-3.9 (-7.1, -0.70)	0.018
AI (P2/P1), % (30 / 27)	-2.5 (15.4)	5.0 (19.3)	-9.4 (-17.3, -1.5)	0.02
AI (AG/PP), % (30 / 27)	-1.6 (7.2)	2.4 (8.9)	-4.4 (-7.9 , -1.0)	0.013
AI@HR75, % (30 / 26)	-0.6 (6.5)	1.8 (9.1)	-3.0 (-6.6 , 0.6)	0.10
Mean ESP, mmHg (30 / 27)	-4.5 (13.8)	2.0 (10.7)	-6.0 (-12.4, 0.4)	0.07
Data are expressed as mean (standard deviation), unless otherwise stated. SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure				

**Table 7-15: BP-PWA parameter change at 12 month follow up,
adjusted for treatment with ACE-I / ARB at baseline**

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
Mean aortic SBP, mmHg	-6.6 (-13.0 , -0.3)	-7.1 (-13.4, -0.7)	0.031
Mean aortic DBP, mmHg	-2.8 (-7.4 , 1.8)	-3.0 (-7.6, 1.6)	0.20
Mean aortic Tr, ms	1.43 (-1.92 , 4.77)	1.42 (-1.99, 4.82)	0.41
Mean augmentation	-3.9 (-7.1, -0.70)	-4.1 (-7.4, -0.9)	0.014
AI (P2/P1), %	-9.4 (-17.3, -1.5)	-9.6 (-17.6, -1.7)	0.019
AI (AG/PP), %	-4.4 (-7.9 , -1.0)	-4.4 (-8.0, -0.9)	0.014
AI@HR75, %	-3.0 (-6.6 , 0.6)	-3.0 (-6.7, 0.6)	0.10
Mean ESP, mmHg	-6.0 (-12.4, 0.4)	-6.3 (-12.7, 0.2)	0.06
Data are expressed as mean (95% confidence interval). SBP, systolic blood pressure; DBP, diastolic blood pressure; Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; ESP, end systolic pressure			

Post hoc analyses

Repeated statistical analysis, including adjustment for the observed change in SUA, did not affect the difference in change in mean central aortic systolic BP observed between treatment groups at either 6 (-2.3mmHg, 95% CI; -9.9, 5.3mmHg, p=0.55) or 12 months (-8.0mmHg, 95% CI; -15.5, -0.4mmHg, p=0.039).

On identifying a statistically significant difference in 12 month change in mean central aortic systolic BP between treatment groups, we performed correlation between baseline SUA and baseline mean central aortic systolic BP and between change in SUA at 6 and 12 months and change in mean central aortic systolic BP at 6 & 12 months. The correlation coefficient and accompanying p value for each correlation are detailed in table 7-16. We identified a non-

significant trend toward a linear correlation between baseline mean central aortic systolic BP and baseline SUA. We identified no correlation between the observed change in SUA and change in mean common CIMT at either 6 or 12 months.

Table 7-16: Central systolic BP correlations with Uric Acid

	Allopurinol	Placebo	All
Baseline	0.136 (0.42)	0.287 (0.077)	0.204 (0.08)
Change at 6 months	-0.083 (0.64)	0.235 (0.19)	0.102 (0.42)
Change at 12 months	0.075 (0.69)	-0.183 (0.37)	0.186 (0.17)
Data are presented as correlation coefficient (p value)			

Figure 7-9: Change in aortic mean systolic BP (mmHg) between baseline and subsequent follow-up

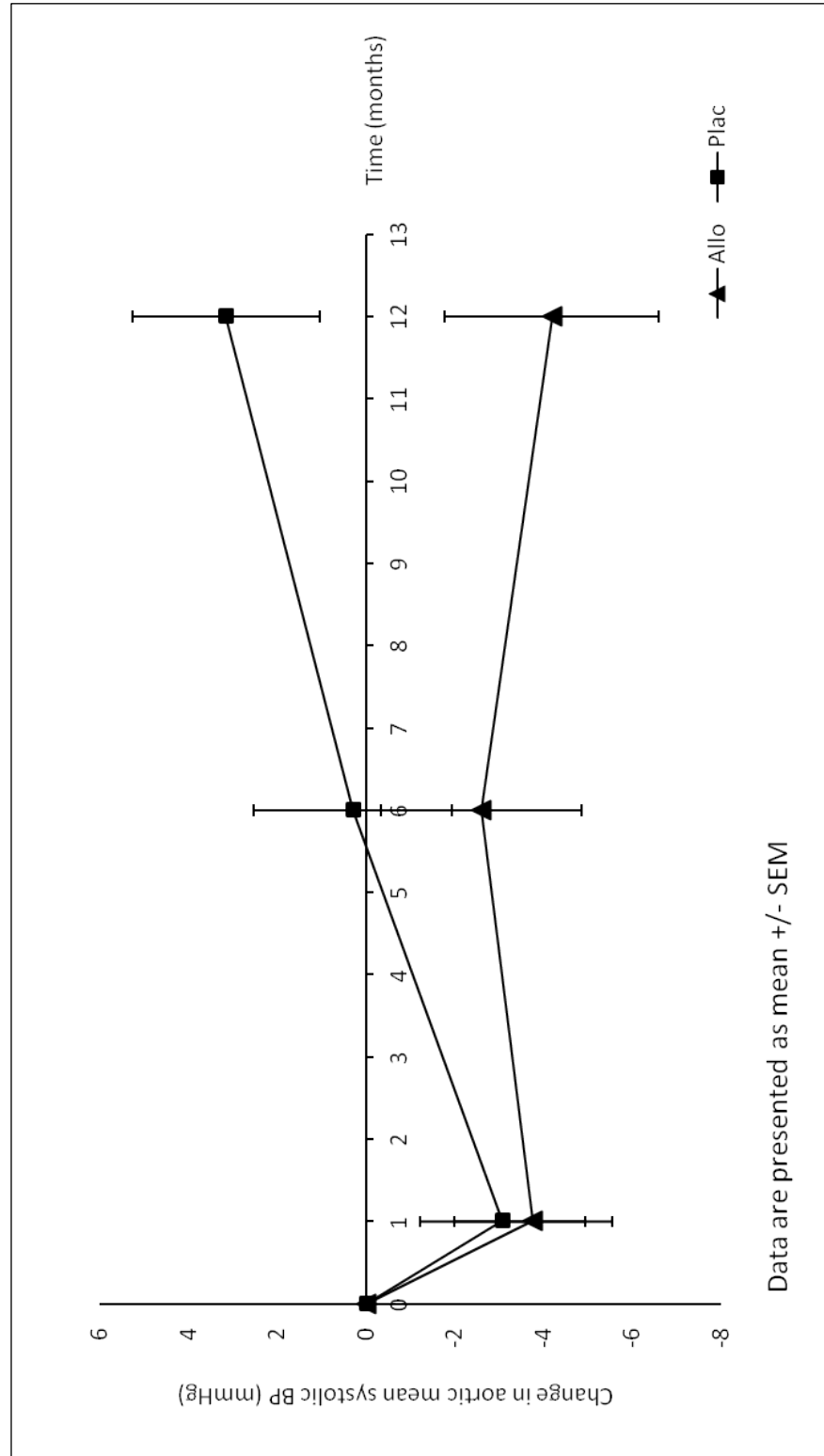
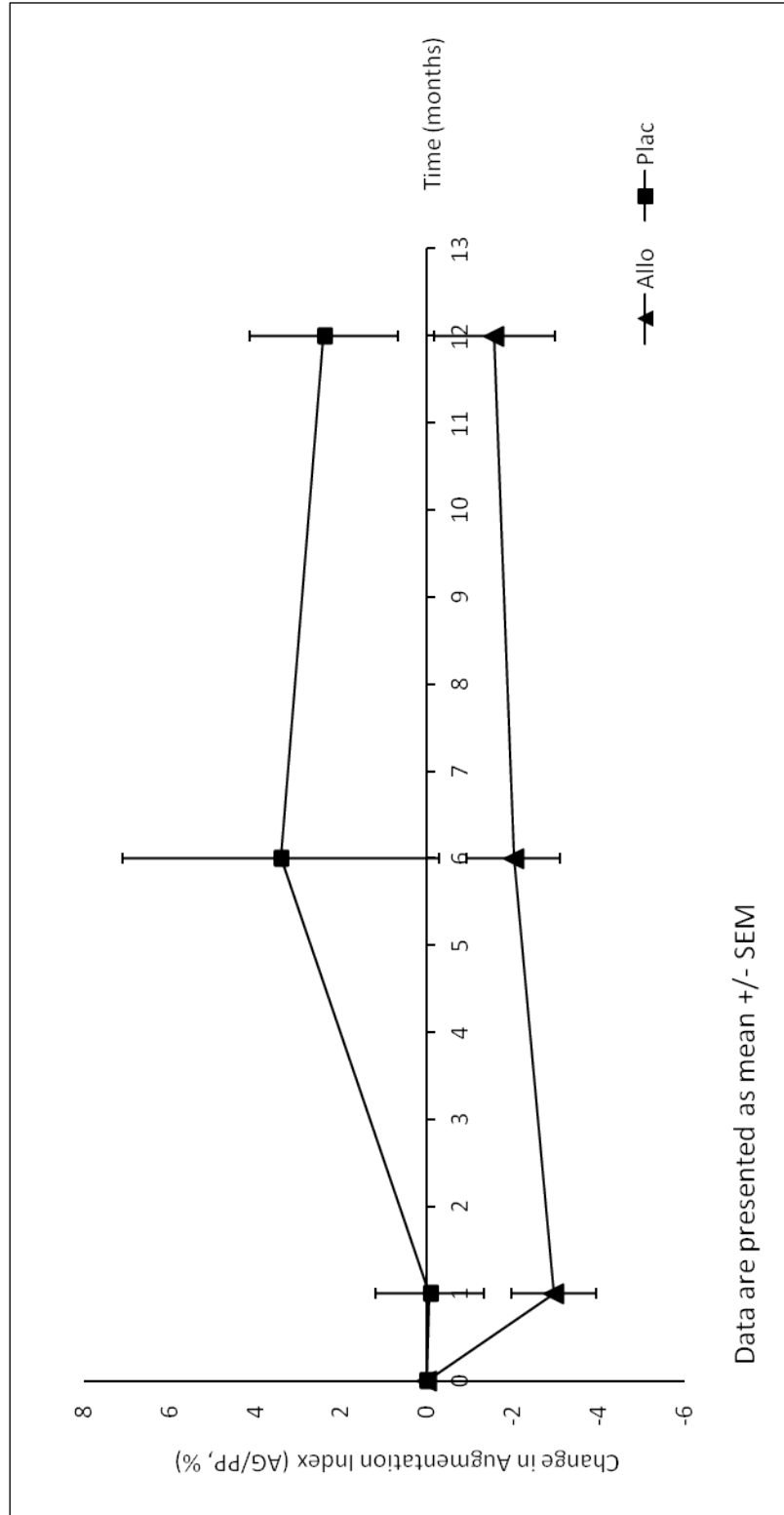


Figure 7-10: Change in Augmentation Index (AG/PP, %) between baseline and subsequent follow-up



BP-PWV Analysis

Baseline BP-PWV values are detailed in table 7-3. 45, 54 and 51 patients had PWV data recorded at both baseline and each of 1, 6 and 12 month visits, respectively, for comparison. Change in BP-PWV parameter values between baseline and 1, 6 & 12 months within each treatment group, together with comparison of intergroup changes from baseline, are detailed in table 7-17. No significant intergroup difference was observed in change in BP-PWV from baseline at any follow up time point. Adjustment for treatment with ACE-I / ARB at baseline did not affect the observed results (table 7-18).

Table 7-17: Change in BP-PWV at 1, 6 & 12 month follow up

Time point (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
1 month (27/19)	-0.03 (0.72)	-0.00 (1.17)	0.00 (-0.06 , 0.06)*	0.91 ¹
6 months (31 / 23)	-0.11 (0.81)	-0.18 (1.23)	0.15 (-0.33, 0.64)	0.53
12 months (23 / 18)	-0.08 (0.97)	-0.07 (1.15)	0.08 (-0.51 , 0.68)	0.78
Data are metres / second, expressed as mean (standard deviation), unless otherwise stated. BP-PWV, blood pressure pulse wave velocity. ANCOVA natural log transformation ¹ .				

Table 7-18: Change in BP-PWV, adjusted for baseline treatment with ACE-I / ARB

Time point (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB)	Adjusted P value
1 month	0.00 (-0.06 , 0.06)*	-0.01 (-0.07 , 0.06)	0.84 ¹
6 months	0.15 (-0.33, 0.64)	0.02 (-0.04 , 0.08)	0.56 ¹
12 months	0.08 (-0.51 , 0.68)	0.01 (-0.06 , 0.08)	0.75 ¹
Data are expressed as mean (95% confidence interval). BP-PWV, blood pressure pulse wave velocity. ANCOVA natural log transformation ¹ .			

BP-brachial Analysis

Baseline brachial BP values are detailed in table 7-3. 74, 71, 72 and 60 patients had brachial BP data recorded at both baseline and each of 1, 3, 6 and 12 month visits, respectively, for comparison.

Change in brachial artery systolic and diastolic BP values, between baseline and 1, 3, 6 & 12 month follow up within each treatment group, together with comparison of intergroup changes from baseline, are detailed in tables 7-19 and 7-20, respectively. Absolute brachial artery systolic and diastolic values at baseline, 1, 3, 6 & 12 months are detailed, respectively, in tables C-2 and C-3 (appendix C). Adjustment for baseline treatment with ACE-I / ARB did not affect the observed results for either systolic (table 7-21) or diastolic (table 7-22) comparisons. Change in brachial artery systolic BP (mmHg) between baseline and subsequent follow-up is illustrated in figure 7-11.

Table 7-19: Brachial artery systolic BP parameter change at 1, 3, 6 & 12 month follow up

Time point (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
1 month (38/36)	-5.9 (15.2)	-3.3 (11.8)	-1.9 (-7.9 , 4.2)	0.54
3 months (35/36)	-1.5 (18.1)	3.7 (16.8)	-4.9 (-12.0 , 2.2)	0.17
6 months (37/35)	-4.5 (17.6)	1.5 (15.2)	-5.4 (-13.1 , 2.2)	0.16
12 months (30/30)	-5.1 (15.8)	5.0 (12.7)	-9.9 (-17.3 , -2.4)	0.01
Data are mmHg, expressed as mean (standard deviation), unless otherwise stated.				

Table 7-20: Brachial artery diastolic BP parameter change at 1, 3, 6 & 12 month follow up

Time point (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
1 month (38/36)	-1.8 (6.3)	-2.4 (9.2)	1.9 (-1.5 , 5.3)	0.28
3 months (35/36)	3.4 (8.7)	5.4 (11.1)	-0.4 (-4.7 , 3.9)	0.85
6 months (37/35)	-2.1 (7.7)	-0.2 (8.9)	-0.9 (-4.7 , 2.9)	0.65
12 months (30/30)	-1.9 (7.8)	0.5 (8.0)	-1.4 (-5.4 , 2.5)	0.47
Data are mmHg, expressed as mean (standard deviation), unless otherwise stated.				

Table 7-21: Brachial artery systolic BP parameter change, adjusted for baseline treatment with ACE-I & ARB

Time point (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
1 month (38/36)	-1.9 (-7.9 , 4.2)	-1.7 (-7.9 , 4.5)	0.59
3 months (35/36)	-4.9 (-12.0 , 2.2)	-6.0 (-13.2 , 1.2)	0.10
6 months (37/35)	-5.4 (-13.1 , 2.2)	-6.1 (-13.9 , 1.7)	0.12
12 months (30/30)	-9.9 (-17.3 , -2.4)	-10.2 (-17.7 , -2.7)	0.09
Data are mmHg, expressed as mean (95% confidence interval).			

Table 7-22: Brachial artery diastolic BP parameter change, adjusted for baseline treatment with ACE- / ARB

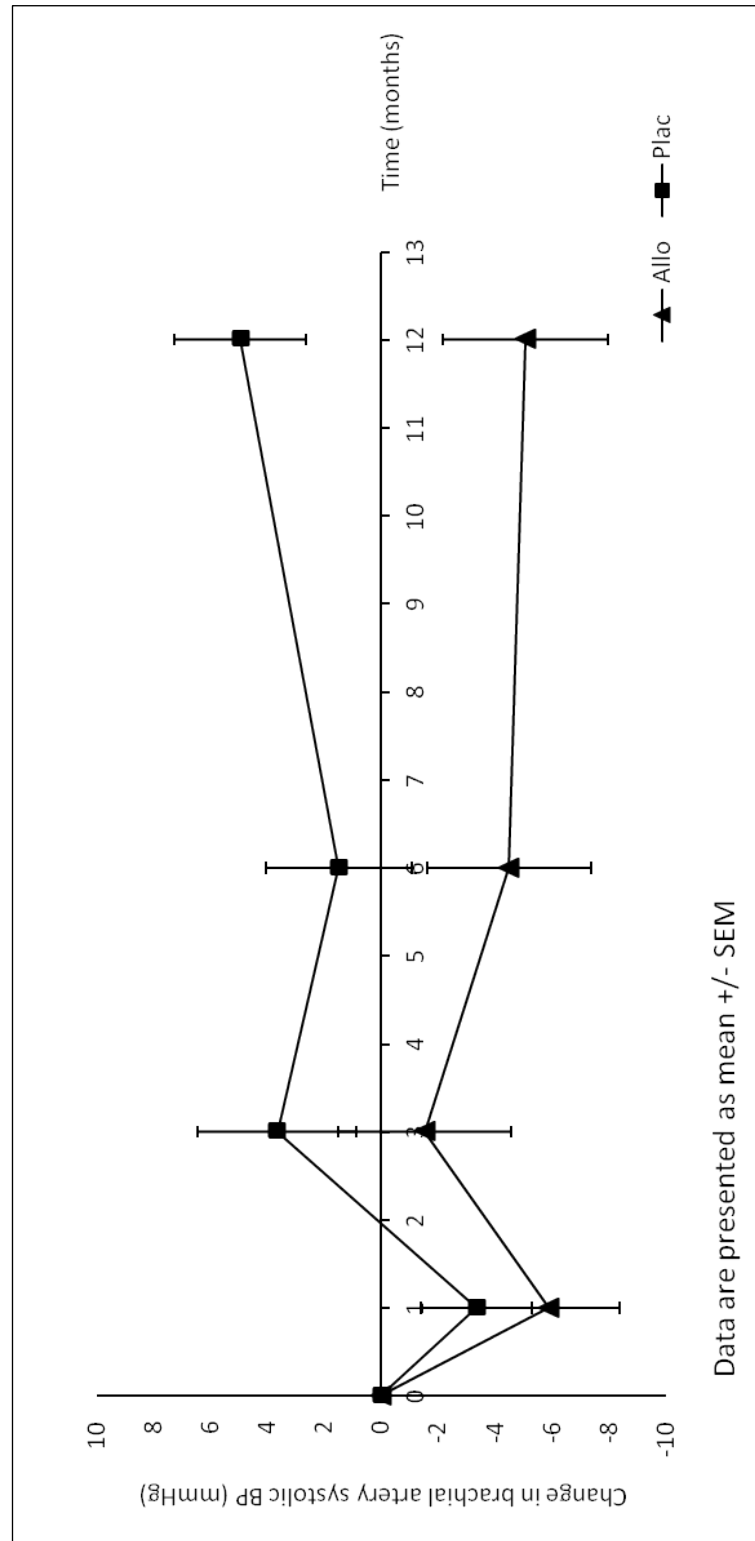
Time point (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I / ARB	Adjusted P value
1 month (38/36)	1.9 (-1.5 , 5.3)	1.7 (-1.8 , 5.1)	0.34
3 months (35/36)	-0.4 (-4.7 , 3.9)	-1.1 (-5.4 , 3.2)	0.60
6 months (37/35)	-0.9 (-4.7 , 2.9)	-0.9 (-4.8 , 3.0)	0.64
12 month (30/30)	-1.4 (-5.4 , 2.5)	-1.6 (-5.5 , 2.3)	0.42
Data are mmHg, expressed as mean (95% confidence interval).			

7.3.3. Peripheral arterial tone reactive hyperaemia index analysis

PAT-RHI values at baseline are detailed in table 7-3. 58 patients had peripheral arterial tone data recorded at both baseline and 1 month visits for comparison. 54 patients had peripheral arterial tone data recorded at both baseline and 6 month visits for comparison. Due to partial technical inadequacy of peripheral arterial tone signal acquisition, determination of RHI was based on 52 patients.

Change in PAT-RHI values at 1 and 6 months, together with intergroup comparison of change from baseline, are detailed in table 7-23. A non-significant trend to reduction in PAT-RHI was observed with allopurinol treatment compared with placebo, at 1 month but was not evident after 6 months. Adjustment for baseline treatment with ACE-I / ARB did not affect the observed results (table 7-24).

Figure 7-11: Change in brachial artery systolic BP (mmHg) between baseline and subsequent follow-up



7.3.4. Circulating markers of vascular biology analysis

Baseline vascular biology values are detailed in table 7-3. The coefficient of variation for the ICAM-1, e-selectin, vWF & thrombomodulin assays were, respectively, 33.7%, 41.7%, 32.6% & 25.4%. 72 patients had circulating markers of vascular biology data recorded at both baseline and 6 month visits for comparison. Explanations for patient unavailability for this analysis are detailed in the CONSORT diagram in figure 7-2.

The distribution of s-ICAM-1 and s-thrombomodulin were positively skewed and therefore a natural logarithmic transformation was used before performing comparative analysis. Change in circulating markers of vascular biology at 6 months, together with intergroup comparison of change from baseline, are detailed in table 7-23. No significant difference between groups was observed for change in any circulating markers of endothelial function at 6 months, including after adjustment for baseline treatment with ACE-I / ARB (table 7-24).

Table 7-23: Endothelial function parameter change at 1 & 6 month follow up

Parameter (n, Allopurinol / Placebo)	Allopurinol	Placebo	Mean intergroup difference, (95% CI)	P value
PAT-RHI				
- 1 month (31/27)	-0.32 (0.83)	0.21 (0.87)	-0.37 (-0.77, 0.04)	0.08
- 6 month (28/24)	-0.19 (1.04)	0.25 (0.76)	-0.21 (0.58, 0.17)	0.27
ICAM-1,ng/ml (37/35)	6.4 (33.3)	6.1 (40.8)	-0.00 (-0.07, 0.07) ¹	0.97 ¹
e-Selectin, ng/ml (37/35)	1.36 (5.95)	-1.77 (6.44)	2.41 (-0.53, 5.35)	0.11
vWF, units/ml (37/35)	0.00 (0.29)	-0.01 (0.33)	-0.03 (-0.11, 0.18)	0.63
Thrombomodulin, ng/ml (37/35)	95 (398)	3 (291)	0.03 (-0.02, 0.07) ¹	0.23 ¹
Data are expressed as mean (standard deviation), unless otherwise stated. PAT-AI, peripheral arterial tonometry augmentation index; PAT-RHI, peripheral artery tonometry reactive hyperaemia index; ICAM-1, inter cellular adhesion molecule-1; vWF, von Willebrand Factor. ANCOVA natural log transformation ¹ .				

**Table 7-24: Endothelial function parameter change at 1 & 6 months,
adjusted for treatment with ACE-I / ARB at baseline**

Parameter (n, Allopurinol / Placebo)	Mean intergroup difference	Adjusted for treatment with ACE-I/ARB	Adjusted P value
PAT-RHI - 1 month - 6 month	-0.37 (-0.77, 0.04) -0.21 (0.58, 0.17)	-0.37 (-0.79, 0.05) -0.25 (-0.63, 0.13)	0.08 0.19
ICAM-1, ng/ml	-0.00 (-0.07, 0.07) ¹	-0.00 (-0.08, 0.07) ¹	0.93 ¹
e-Selectin, ng/ml	2.41 (-0.53, 5.35)	2.17 (-0.81, 5.15)	0.15
vWF, units/ml	-0.03 (-0.11, 0.18)	0.03 (-0.12, 0.17)	0.69
Thrombomodulin, ng/ml	0.03 (-0.02, 0.07) ¹	0.03 (-0.02, 0.07) ¹	0.25 ¹
Data are expressed as mean (95% confidence interval). PAT-AI, peripheral arterial tonometry augmentation index; PAT-RHI, peripheral artery tonometry reactive hyperaemia index; ICAM-1, inter cellular adhesion molecule-1; vWF, von Willebrand Factor. ANCOVA natural log transformation ¹ .			

Cardiovascular events

There was no difference between Allopurinol and Placebo treatment groups for recurrent stroke, TIA, MI or any combination of these clinical endpoints (table 7-25).

Table 7-25: Cardiovascular events at 12 month follow-up

	Allopurinol N (%)	Placebo N (%)	Intergroup difference %, (95% CI)	P value
Combined recurrent TIA / stroke / MI	3 (7.5%)	4 (10.0%)	-2.5% (-14.9, 9.9%)	0.69
Analysis by difference in two proportions. TIA, transient ischaemic attack; MI, myocardial infarction.				

7.4. Discussion

This double blind randomised controlled trial found that one year of treatment with allopurinol reduced mean common CIMT progression, central systolic BP and arterial stiffness, in patients with recent ischaemic stroke or TIA. No differences in either mean maximum CIMT progression or measures of endothelial function were observed. These results represent the first observation that allopurinol can favourably modify CIMT progression and central BP and extends the evidence in the stroke population to include sustained treatment effects in relation to arterial stiffness. These data provide evidence in support of the hypothesis that XO inhibition may reduce the risk of cardiovascular events.

CIMT progression

As discussed in chapters 4 and 5, there is a mechanistic basis for XO inhibition having favourable effects on CIMT. UA holds numerous molecular and cellular properties which may contribute to atherogenesis, including SMC proliferation³⁵³ and LDL oxidation³⁴⁵. In addition, XO activity, through elevated oxidative stress and in turn endothelial dysfunction, holds atherogenic properties³⁷⁶. As detailed in Chapter 5, in addition to reducing SUA, XO inhibition has been shown to favourably modify both oxidative stress and endothelial dysfunction. XO inhibition has also been shown to reduce BP in adolescent hypertensives³⁵⁹ and as shown in this study, appears to confer favourable central haemodynamic effects in the stroke population.

The CIMT progression findings in the current study necessitate a degree of caution in interpretation. Firstly, this was a small study with insufficient power to exclude a potentially important treatment effect. Despite this, a statistically significant treatment effect in favour of Allopurinol was observed. Whilst the annualised CIMT progression rate in the placebo group exceeded that included in the power calculation, it was within 95% confidence intervals based on other population statistics. Nevertheless, reproduction of the current finding in a separate population is needed to provide assurance that these findings do not represent a type 1 statistical error.

Secondly, benefit was observed only on mean common CIMT and not on mean maximum CIMT progression. This may be accounted for by the observation that mean maximum CIMT incorporates CIMT measurements at multiple carotid segment levels and represents an inherently more variable measurement parameter (coefficient of variation in the study population was 26% for mean maximum CIMT compared with 18% for mean common CIMT), making it a less powerful outcome measure⁵²⁰. It is now accepted that mean common CIMT is the recommended standard for reporting in both clinical practice and research studies^{520, 551}. The within observer intra-class correlation coefficient for mean common CIMT was 0.87 (95% CI, 0.77 – 0.93) and for mean maximum CIMT was 0.91 (95% CI, 0.79 to 0.96), both of which appeared satisfactory. The study demonstrated feasibility of CIMT measurement in the local population, provides data regarding CIMT and annual CIMT progression in the local ischaemic stroke and TIA population to inform a power calculation for future study and the data may be included in any future meta-analysis.

Evidence which has emerged following the inception of this study now suggests that CIMT progression may have limitations as a surrogate marker for modified CV risk in clinical trials. Both CIMT and CIMT progression have been shown to independently predict risk of subsequent cardiovascular events, including stroke^{526, 527}. In the case of the latter, there is now debate as to whether progression independently predicts risk or whether retardation in CIMT progression by therapeutic means modifies cardiovascular risk, independently of other mechanisms^{547, 548}. This may reflect lack of sensitivity rather than change in CIMT not reflecting CV risk. Nonetheless, the observation in this study that CIMT progression was retarded with active treatment is encouraging when considering its potential in prevention of atherosclerotic cardiovascular disease.

Central blood pressure, arterial stiffness and endothelial function

Epidemiological studies have demonstrated an independent association between UA and incident hypertension³⁵⁸. Both UA and the ROS produced through XO activity hold properties which are implicated in the pathogenesis and pathophysiology of hypertension. Uric acid exerts effects within the macula densa³⁵⁴, appears to influence renal salt

handling³⁵⁶ and exerts effects on the Renin angiotensin system³⁵⁷. Xanthine oxidase is likely to represent a significant source of vascular oxidative stress, particularly under pathophysiological conditions³⁷⁷. This is associated with impaired endothelial function which is in turn associated with impaired balance of production of vasodilatory and vasoconstrictive agents including NO and endothelin^{346, 376}. In addition, XO activity appears to be inter-related with angiotensin II^{377, 378}, which is part of the final common pathway in initiating hypertensive change with the vasculature⁶³⁶. In recent years, evidence has emerged that Allopurinol can reduce systemic BP. In an adolescent population with hypertension naive to other antihypertensive therapy, 1 month of treatment with Allopurinol resulted in a reduced BP of 6.9 /2.0 mmHg³⁵⁹. Subsequently, the same group demonstrated that in a similar population, uric acid reduction *per se*, achieved through treatment with either Allopurinol or Probenecid, was effective in reducing blood pressure⁶³⁷. Studies examining BP lowering effects in other hypertensive populations have not been forthcoming.

More recently, several studies have been reported which included indirect measures of haemodynamic effects of allopurinol. In populations with CRF⁶³⁸, CHD⁶³⁰ and DM²²⁴, Allopurinol has been found to reduce left ventricular mass in patients with evidence of left ventricular hypertrophy. Although LVH is considered to reflect cardiac after load and systemic BP, the authors of these studies observed that the reduction in LV mass appeared to occur independently of any treatment effect on BP (though the studies may have lacked power to demonstrate a BP lowering effect *per se*). This has raised the question as to whether Allopurinol has the potential to reduce BP in these populations but also as to how Allopurinol confers favourable effects on LV mass if this is not through reduction in systemic BP.

Several potential mechanisms of BP independent improvement in LV mass have been proposed. Firstly, reduced arterial stiffness may reduce cardiac afterload, even if brachial artery BP is unchanged⁶³⁸. Secondly, Allopurinol has been shown to reduce oxygen consumption by the myocardium⁶³⁹ and reduces oxidative stress which may play a role in left ventricular hypertrophy⁶⁴⁰. In addition, Allopurinol appears to confer anti-ischaemia effects. In patients with angina, treatment was associated with improved exercise capacity and time to ST depression on ECG monitoring⁴³⁹.

The data in the current study may provide an additional mechanism which could account for findings in respect of LV mass. A significant reduction in central mean systolic BP, derived through BP-PWA, was observed with 12 months of Allopurinol therapy. Central BP measurement may represent a better predictive tool than brachial BP for risk of cardiovascular events ^{588, 589}, which may reflect central BP more accurately reflecting cardiac afterload and the pressure to which coronary and cerebral arteries are exposed.

Pleiotropic effects of BP agents with apparent superiority have been proposed as underlying these findings. Clinical trials which have demonstrated differential reduction in risk of CV events between antihypertensive agents, despite similar reductions in brachial BP ⁵⁹¹, have also observed differential effects on central BP between agents, proposing this as a potential mechanism which might account for differential effects on risk. Thus, the current findings of central BP reduction offer a potential mechanism by which LV mass might be reduced with Allopurinol. In addition, a significant reduction in brachial systolic BP was observed in the current study following 12 months treatment. A non-significant trend to reduction in brachial SBP was observed at 1, 3 and 6 months. At 12 months the magnitude of this difference was estimated at nearly 10 mmHg, though 95% confidence intervals were wide. The observed statistically significant difference at 12 months was partly attributable to a rise BP in the placebo group that exceeded that which would usually be expected in a clinical trial population. A clinical trial of the effect of Allopurinol on 24-hour ambulatory BP measures should be performed to confirm the effect on blood pressure.

Augmentation index was significantly reduced with allopurinol therapy. AI, standardised for a heart rate of 75 bpm was non-significantly reduced. It has been suggested that this parameter may represent a better measure for AI reporting ⁶⁴¹, though the epidemiological association with risk of cardiovascular events is established for uncorrected AI ⁶⁴². In a small study with three month follow up, allopurinol treatment was previously associated with a reduction in AI ⁴²⁷. The current study results confirm this finding in a larger sample and provide evidence of a sustained treatment effect.

The reduction in central BP may be attributable to this accompanying observed reduction in arterial stiffness, which represents a key determinant of central BP ⁵⁸⁰. In turn, endothelial function is a determinant of arterial stiffness ⁵⁶³. In patients with cardiovascular disease,

large artery endothelial function is improved with allopurinol therapy⁶²⁷ which also improves endothelial NO bioavailability⁴¹⁵, offering a potential mechanism for reduced arterial stiffness following allopurinol treatment. Of note, carotid - radial PWV was not affected by Allopurinol therapy in the current study. Considered together with the AI findings, effects on arterial stiffness may be conferred through greater effects on larger artery function relative to resistance vessels.

No significant benefit on endothelial function, determined either as PAT-RHI or circulating markers of endothelial activation, was observed in the current study. This was somewhat unexpected and at odds with the otherwise beneficial effects observed. The study may have lacked statistical power for this outcome, as the PAT RHI outcome measurement was included only after the study commenced, limiting the number of participants available for analysis of this endpoint. In addition, PAT RHI represents a composite of both large and small arterial function. Although both large artery and resistance vessel endothelial function are each correlated with coronary endothelial function, the two measures are themselves less well correlated⁶¹⁹. Reported as an index of pulse wave amplitude, lack of benefit in the EndoPAT measurement could potentially reflect reduced vascular tone in the context of improved endothelial function overall. However, the current study lacked control measures to further evaluate this hypothesis.

Circulating markers of vascular inflammation and endothelial function were also unchanged following 6 months of treatment. In a previous study of Allopurinol effects in a stroke population, 2 months of treatment was associated with a reduction in ICAM-1 compared with Placebo therapy⁴¹⁴. This result appeared to be driven by an attenuation of rise in ICAM-1 in the Allopurinol group following stroke. It may be that Allopurinol confers beneficial effects with respect to the initial propensity to endothelial activation in the period immediately following an ischaemic event but that after a 6 month interval, endothelial activation has returned to baseline.

Relative importance of UA reduction in mediating beneficial effects of XO

Previous studies in patients with heart failure suggest the beneficial effects of allopurinol may be independent of any reduction in SUA ²⁹⁹. The current study utilised only one UA lowering therapeutic strategy and no measure of oxidative stress. It was therefore not designed to further differentiate the mechanism of benefit of Allopurinol. However, in post-hoc analysis, no correlation between the observed change in UA and change in either mean common CIMT or central BP was identified, potentially indicating a UA independent treatment effect. Statistical analysis with adjustment for the observed change in UA did not affect the observed results for either CIMT or central BP outcomes.

Study participants were enrolled irrespective of their baseline UA level. Consequently, although highly statistically significant, the observed therapeutic reduction in UA level (0.08 mmol/l) was relatively modest, and from a lower baseline level (0.31 mmol/l), compared with studies which selected patients based on elevated serum UA. Baseline uric acid did not differ significantly between patients treated with known uricosuric agents and those who were not (SUA 0.33mmol/l vs. 0.30mmol/l, $p = 0.10$). *Post hoc* analysis of the largest trial of XO inhibition to date, the OPT-CHF study, suggested benefit of XO inhibition may be limited to those with baseline hyperuricaemia ³⁹⁸, with no difference observed between active treatment and placebo groups in the trial as a whole. Conversely, improved endothelial function has been reported with XO inhibition in patients with increased cardiovascular risk despite apparently normal levels of UA ⁴³⁴. It is feasible that patient subgroups exist who might experience differential benefit of XO, according to their baseline UA level. Through inclusion of patients irrespective of baseline UA level, the results of this study provide evidence of treatment benefit in a general population with normal range uricaemia.

Safety profile

The study provides reassurance regarding the safety profile of allopurinol in this population: treatment dose was well tolerated during the study, with high concordance documented through pill counts and confirmed with the observed reduction in serum UA. No serious adverse reactions attributable to active study medication were observed.

Limitations

Although the study was a double blind RCT, thereby minimising likelihood of bias, there were several limitations which must be considered. Firstly, as discussed above, the study lacked power to detect a CIMT progression treatment effect between groups. Progression in the placebo group was higher than anticipated and the CIMT findings require reproduction in a separate population.

Secondly, the study utilised only one UA lowering strategy and was therefore not designed to further differentiate the mechanism of benefit in terms of UA lowering versus reduced vascular oxidative stress.

Thirdly, despite randomisation, there were minor imbalances between treatment groups. These included a greater proportion of participants in the placebo group with index event of stroke (rather than TIA), diabetes and beta-blocker treatment, whilst fewer had documented hypertension, AF or treatment with ACE-I / ARB agents. The difference in the proportion of patients treated with ACE-I or ARB between treatment groups was significant and potentially favoured the observation of a treatment effect in favour of allopurinol. Therefore, statistical analysis with adjustment for this imbalance was performed and found the treatment effect on CIMT progression, central mean systolic pressure, brachial systolic BP and AI, were each unchanged. There was no significant difference between the treatment groups in modification of any therapy associated with reduced risk of cardiovascular events during the 12 months of follow up. It is feasible that other unidentified confounding factors could have influenced the observed results.

Fourthly, there was incomplete data available for all study outcomes at final follow up visits. This predominantly reflected patient withdrawals but in a proportion of cases this was attributable to technical inadequacy of measurements, particularly in the case of BP-PWA and BP-PWV outcomes. Consequently, some degree of attrition bias may have been introduced. However, patient withdrawals were unrelated to the study intervention and incomplete availability of final follow up endpoint data was balanced between the two treatment groups. Baseline endpoint parameter values in the randomised population did not differ significantly from those in the population with data available for 12 month analysis.

Final endpoint follow up data was available in 94%, 83%, 87% and 88% of patients for CIMT, BP-PWA, brachial BP and endothelial function, respectively.

Fifthly, this study did not explore whether a dose dependent response exists with treatment. It is feasible that greater magnitude of benefit than that observed in the current study could be achieved with allopurinol therapy; a dose-dependent improvement in endothelial function has been reported²⁹⁹ and regression in LVH was achieved with higher doses than were used in this trial⁶³⁰.

Finally, the study population represented a group of patients with either predominantly mild severity ischaemic stroke or TIA, who were able to participate in repeated study visits over a 12 months period. The population was neither restricted nor enriched according to stroke aetiological criteria. As such, the results may not be generalisable to the entire ischaemic stroke population.

Conclusion

In conclusion, this randomised, placebo-controlled double blind study extends the evidence of sustained beneficial effects of allopurinol treatment on the vasculature to include the stroke patient population, with effects including reductions in CIMT progression, central BP and arterial stiffness. No treatment effect was observed in relation to studied measures of endothelial function. These data support the hypothesis that XO inhibition holds the potential to reduce the risk of cardiovascular events and provide evidence supportive of further evaluation of this therapeutic strategy in definitive clinical endpoint studies.

Chapter 8

Final conclusions & future directions

Despite the emergence of effective treatments for acute stroke, incomplete effectiveness and applicability together with high incidence, mean that cerebrovascular disease remains a leading cause of both mortality and adult disability. The burden of cerebrovascular disease is set to rise with the effects of globalisation and expansion of the elderly population. Preventative strategies are crucial in limiting this burden.

The studies included in this thesis address two aspects of secondary prevention following ischaemic stroke and TIA; firstly, the potential for optimised detection of paroxysmal AF and in turn, appropriate use of anticoagulant based secondary prevention; secondly; the potential for a novel therapeutic strategy in the prevention of cardiovascular and cerebrovascular disease; xanthine oxidase inhibition.

In a randomised controlled trial, including unselected patients with acute ischaemic stroke or TIA, the routine addition of 7 days non-invasive cardiac event monitoring to current guideline based clinical practice, was found to significantly enhance detection of episodes of paroxysmal AF which would justify initiation of anticoagulant therapy. Treatment with anticoagulation was also significantly enhanced. In addition, cardiac event monitoring detected a high proportion of patients in this population with evidence of briefer episodes of PAF, the significance of which is uncertain, as is the appropriateness of more aggressive treatment with anticoagulation. This study provides the first randomised evidence, to guide clinicians in the approach to investigation for initially occult paroxysmal AF in unselected patients with ischaemic stroke and TIA. Clinical practice based on existing clinical guidelines appears inadequate and this study should allow the level of evidence and strength of recommendations of future guidelines to be improved.

In a related observational study, the PPV of AF detection in the immediate aftermath of ischaemic stroke and TIA, for AF subsequently evident after a 90 day interval, was found to be high. This suggests that AF detected through early monitoring is unlikely to represent a transient phenomenon, attributable to transient physiological conditions associated with acute ischaemia. Accordingly, treatment decisions based on AF identification in this period appear justified. Negative predictive value of early monitoring for subsequent AF detection was only moderate, raising the question as to whether repeated interval monitoring might be justified in selected patients. This question, together with clarification of the relevance of

brief episodes of AF detectable early after stroke, should form the basis for future research in this area.

A systematic review and meta-analysis confirmed that XO inhibitors confer beneficial effects within the vasculature, including improved endothelial function and reduced circulating markers of oxidative stress. Whilst evidence is available which supports the hypothesis that XO inhibitors have the potential to reduce the risk of cardiovascular events, clinical endpoint studies are lacking and studies demonstrating beneficial effects on surrogate markers of outcome have tended to be of limited duration. The optimal dose and target population for treatment remains uncertain, as does the mechanism of benefit.

In a double blind, randomised, placebo controlled trial, which included patients with recent ischaemic stroke or TIA, one years' treatment with the XO inhibitor Allopurinol was found to reduce mean common CIMT progression, central systolic BP and arterial stiffness. Treatment was well tolerated, with no treatment related serious adverse reactions. Observed beneficial treatment effects may have been unrelated to the observed reduction in UA, though further study is required to fully differentiate underlying mechanisms. No treatment effect was observed in relation to studied measures of endothelial function. The study extended the evidence of XO inhibitor treatment effects to sustained effects on arterial stiffness within the stroke patient population and to surrogate measurements of CIMT progression and central BP. These data support the hypothesis that XO inhibitors hold the potential to reduce cardiovascular events and provide evidence supportive of further evaluation of this therapeutic strategy in definitive clinical endpoint studies.

In this regard, a randomised double blind placebo controlled trial is planned which will evaluate Allopurinol in patients with ischaemic stroke or TIA in relation to each of; white matter hyperintensities and silent brain infarction (on MRI); LVH regression; peripheral arterial BP and clinical outcomes, including both measures of cognition and rates of cardiovascular events. The study aims to definitively establish whether allopurinol has beneficial effects on three powerful surrogate endpoints for adverse outcomes after stroke and thereby allow a pivotal large scale clinical endpoint study to be designed, funded and implemented.

Appendix A

Additional data tables relating to Chapter 3

Table A-1: Sensitivity, specificity, PPV & NPV for SP investigation detected AF at 90 days, in the SP, SP-AM and combined study groups, for corresponding duration AF episodes after 90 days

	Sensitivity	Specificity	PPV	NPV
SP group 90 “Sustained” PAF	29 (4 - 71)	95 (74 - 100)	67 (9 - 99)	78 (56 - 93)
SP group 90 “any duration” PAF	19 (4 - 46)	100 (74 - 100)	100 (37 - 100)	44 (23 - 66)
SP-AM group 90 “Sustained” PAF	57 (18 – 90)	88 (70 – 100)	80 (28 – 100)	83 (59 – 96)
SP-AM group 90 “any duration” PAF	43 (18 – 71)	89 (52 – 100)	86 (42 – 100)	50 (25 – 75)
Combined group 90 “Sustained” PAF	43 (18 - 71)	94 (81 - 99)	75 (35 - 97)	81 (65 - 91)
Combined group 90 “any duration” PAF	30 (15 - 49)	95 (74 - 100)	90 (56 - 100)	46 (30 - 63)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table A-2: Sensitivity, specificity, PPV & NPV for all combined Study 1 investigations at 90 days, in the SP-AM and combined study groups, for corresponding duration AF episodes after 90 days

	Sensitivity	Specificity	PPV	NPV
SP-AM Group 90 “Sustained” PAF	81 (29 - 96)	94 (70 - 100)	83 (36 - 100)	88 (64 - 99)
SP-AM Group 90 “any duration” PAF	79 (29 - 96)	89 (52 - 100)	92 (62 - 100)	73 (39 - 94)
Combined Group 90 “Sustained” PAF	50 (23 – 77)	94 (81 – 99)	78 (40 – 97)	83 (67 – 93)
Combined Group 90 “any duration” PAF	47 (28 – 66)	95 (74 – 100)	93 (68 – 100)	53 (35 – 70)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table A-3: Sensitivity, specificity, PPV & NPV of “any duration” PAF, on SP investigations at 90 days, in the SP, SP-AM and combined study groups, for “sustained” duration PAF on interval R-test investigation after 90 days

	Sensitivity	Spec	PPV	NPV
SP-AM Group SP 90	71 (29 – 96)	88 (62 – 98)	71 (29 – 96)	88 (62 – 98)
SP Group SP 90	29 (4 – 71)	95 (74 – 100)	67 (9 – 99)	78 (56 – 93)
Combined Group SP 90	50 (23 – 77)	91 (77 – 98)	70 (35 – 93)	82 (67 – 93)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Table A-4: Sensitivity, specificity, PPV & NPV of “any duration” PAF, on all combined Study 1 investigations at 90 days, in the SP-AM and combined study groups, for “sustained” duration PAF on interval R-test investigation

	Sensitivity	Spec	PPV	NPV
SP-AM Group initial R & SP 90	100 (65 – 100)	69 (41 – 89)	58 (28 – 85)	100 (76 – 100)
Combined Group R & SP 90	64 (35 – 87)	83 (66 – 93)	60 (32 – 84)	85 (69 – 95)
Data are presented as % (95% CI). SP, standard practice; SP-AM, standard practice plus additional monitoring; PAF, paroxysmal atrial fibrillation; PPV, positive predictive value; NPV, negative predictive value				

Appendix B

Additional data tables & figures relating to Chapter 5

Table B-1: Sensitivity analysis for brachial artery FMD studies

Study removed	I ²	Mean Difference (95% CI)	P value
Doehner 2002	82%	2.22 (-0.42, 4.86)	0.10
Guthikonda 2004	81%	2.07 (-0.42, 4.55)	0.10
El Solh 2006	82%	2.43 (-0.46, 5.31)	0.10
Eskurza 2006	0%	3.36 (2.00, 4.71)	<0.00001
Yiginer 2008	81%	2.43 (-0.51, 5.37)	0.11
FMD data are expressed as a % change in vessel diameter			

Table B-2: Sensitivity analysis for venous occlusion plethysmography FBF studies

Study removed	I ²	Mean Difference (95% CI)	P value
O'Driscoll 1999	6%	84.80 (54.55, 115.05)	<0.00001
Butler 2000	64%	55.83 (-5.78, 117.43)	0.08
Farquharson 2002	71%	72.13 (2.14, 142.11)	0.04
Guthikonda 2003	64%	60.66 (11.33, 109.98)	0.02
George 2006	71%	67.11 (-9.27, 143.50)	0.09
FBF data are expressed as % change in flow relative to non-infused control arm			

Table B-3: Sensitivity analysis for MDA studies

Study removed	I ²	Mean Difference (95% CI)	P value
Heunks 1999	92%	-0.53 (-0.85, -0.20)	0.001
Butler 2000	93%	-0.75 (-1.21, -0.30)	0.001
Farquharson 2002	92%	-0.48 (-0.79, -0.17)	0.002
Desco 2002	93%	-0.75 (1.24, -0.26)	0.003
El Solh 2006	94%	-0.64 (-1.01, -0.27)	0.0006
Yiginer 2008	86%	-0.34 (-0.57, -0.12)	0.003
MDA data are expressed as nmol/ml			

Figure B-1: Forrest plot detailing end of treatment phase brachial artery FMD (% change in vessel diameter); individual study data

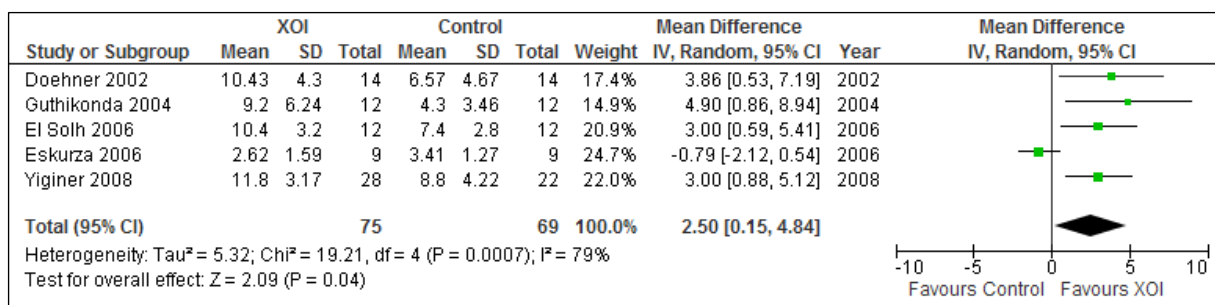


Figure B-2: Forrest plot detailing end of treatment phase FBF response to ACh (% change in flow relative to non-infused control arm); individual study data

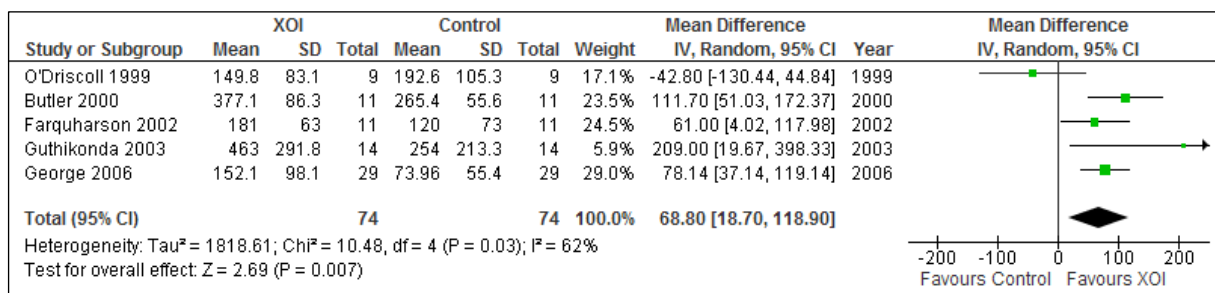
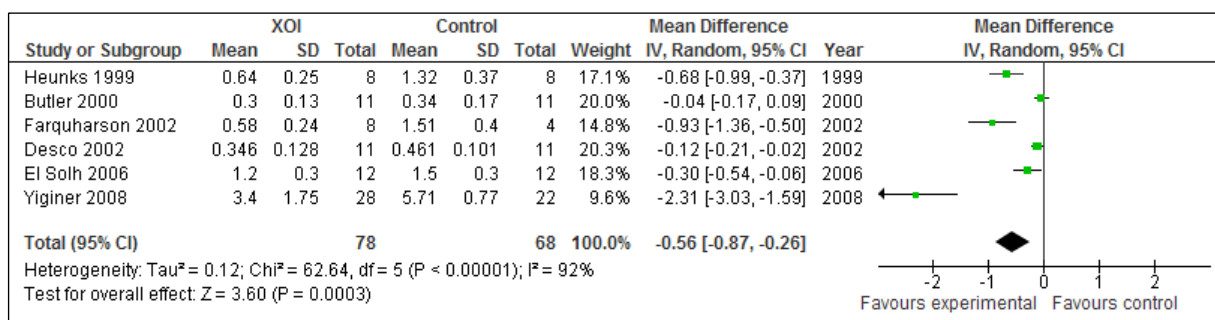


Figure B-3: Forrest plot detailing end of treatment phase circulating MDA (nmol/ml); individual study data



Appendix C

Additional data tables & figures relating to Chapter 7

Table C-1: Baseline outcome parameter values for subgroups with complete data available for interval comparison

Study sample, n	Allopurinol	Placebo	All
Uric acid, mmol/l			
Randomised, 37 / 40	0.32 (0.09)	0.31 (0.08)	0.32 (0.09)
1 month analysis, 33 / 36	0.32 (0.10)	0.30 (0.08)	0.31 (0.09)
3 month analysis, 34 / 37	0.32 (0.10)	0.30 (0.08)	0.31 (0.09)
6 month analysis, 34 / 35	0.31 (0.09)	0.30 (0.08)	0.31 (0.09)
12 month analysis, 34 / 34	0.32 (0.09)	0.30 (0.08)	0.31 (0.09)
Mean maximum CIMT, mm			
Randomised, 39 / 40	1.20 (0.32)	1.17 (0.30)	1.18 (0.31) (95% CI, 1.111, 1.249)
6 month analysis, 35 / 32	1.21 (0.33)	1.20 (0.30)	1.20 (0.32) (95% CI, 1.122, 1.278)
12 month analysis, 31 / 34	1.18 (0.25)	1.16 (0.32)	1.17 (0.29) (95% CI, 1.098, 1.241)
6 months sensitivity analysis, 34/31	1.22 (0.33)	1.20 (0.31)	1.21 (0.32) (95% CI, 1.131, 1.289)
Mean common CIMT (far wall), mm			
Randomised, 39 / 40	0.83 (0.16)	0.82 (0.15)	0.83 (0.15) (95% CI, 0.796, 0.864)
6 month analysis, 35 / 32	0.84 (0.16)	0.84 (0.15)	0.84 (0.16) (95% CI, 0.801, 0.879)
12 month analysis, 31 / 34	0.85 (0.17)	0.82 (0.16)	0.83 (0.16) (95% CI, 0.790, 0.870)
12 months sensitivity analysis, 31 / 32	0.85 (0.17)	0.82 (0.16)	0.84 (0.16) (95% CI, 0.780, 0.880)
Mean aortic SBP, mmHg			
- Randomised (40 / 39)	112.3 (15.79)	111.0 (14.03)	111.7 (14.86)
- 1 month CG (32/28)	112.00 (16.64)	110.40 (10.36)	111.26 (13.97)
- 6 month CG (35 / 33)	111.99 (16.09)	108.55 (10.98)	110.32 (13.85)
-12 month CG (30 / 27)	110.65 (15.25)	107.60 (11.64)	109.21 (13.63)

Mean aortic DBP, mmHg			
- Randomised (40 / 39)	91.0 (10.59)	88.9 (9.59)	90.0 (10.10)
- 1 month CG (32/28)	91.34 (10.56)	89.43 (6.47)	90.45 (8.87)
- 6 month CG (35/33)	90.90 (10.76)	87.92 (7.48)	89.45 (9.36)
-12 month CG (30/27)	112.71 (15.27)	110.85 (12.21)	111.83 (13.82)
Mean aortic Tr, ms			
- Randomised (40 / 38)	136.4 (9.43)	135.2 (10.67)	135.8 (10.0)
- 1 month CG (32/26)	137.18 (9.58)	135.26 (12.15)	136.32 (10.75)
- 6 month CG (35 / 32)	136.32 (9.82)	135.68 (11.21)	136.01 (10.43)
-12 month CG (30 /26)	136.54 (10.35)	134.15 (5.80)	135.43 (8.55)
Mean augmentation, mmHg			
- Randomised (40/39)	17.3 (7.52)	18.5 (8.26)	17.9 (7.87)
- 1 month CG (32/28)	16.97 (7.75)	17.76 (7.33)	17.34 (7.50)
- 6 month CG (35/33)	17.36 (7.45)	17.19 (7.14)	17.28 (7.25)
-12 month CG (30/27)	17.01 (7.42)	17.56 (7.05)	17.27 (7.19)
AI (P2/P1), %			
- Randomised (40/39)	152.0 (15.03)	155.4 (20.3)	153.7 (17.79)
- 1 month CG (32/28)	152.74 (14.96)	154.87 (20.39)	153.73 (17.58)
- 6 month CG (35/33)	152.59 (14.85)	154.32 (19.95)	153.43 (17.40)
-12 month CG (30/27)	151.62 (15.54)	155.21 (20.86)	153.32 (18.18)
AI (AG/PP), %			
- Randomised (40/39)	33.9 (7.02)	34.6 (8.45)	34.2 (7.71)
- 1 month CG (32/28)	33.85 (7.13)	34.36 (8.59)	34.09 (7.78)
- 6 month CG (35/33)	34.20 (7.00)	34.16 (8.45)	34.18 (7.68)
-12 month CG (30/27)	33.74 (7.41)	34.48 (8.70)	34.09 (7.98)
AI@HR75, %			
- Randomised (40/39)	28.4 (8.05)	29.3 (7.87)	28.8 (7.93)
- 1 month CG (32/28)	27.95 (8.21)	29.35 (7.85)	28.60 (8.00)
- 6 month CG (35/33)	28.50 (8.28)	28.83 (8.04)	28.66 (8.10)
-12 month CG (30/26)	28.30 (8.85)	29.38 (8.14)	28.80 (8.47)
Mean end systolic pressure, mmHg			
- Randomised (40/39)	114.5 (16.03)	114.3 (14.92)	114.4 (15.39)
- 1 month CG (32/28)	114.33 (16.60)	113.94 (10.45)	114.15 (13.96)
- 6 month CG (35/33)	114.26 (16.43)	111.74 (11.59)	113.03 (14.24)
-12 month CG (30/27)	112.71 (15.27)	110.85 (12.21)	111.83 (13.82)
PWV (brachial), m/s			
- Randomised (35/33)	8.55 (1.01)	8.41 (0.96)	8.48 (0.98)
- 1 month CG (27/19)	8.54 (1.02)	8.65 (0.80)	8.58 (0.93)
- 6 month CG (31/23)	8.58 (1.00)	8.42 (1.09)	8.51 (1.03)
-12 month CG (23/18)	8.42 (0.96)	8.25 (0.97)	8.34 (0.96)

Brachial SBP, mmHg			
- Randomised	135.3 (20.30)	135.2 (18.39)	135.2 (19.24)
- 1 month CG	134.61 (20.33)	131.73 (14.97)	133.21 (17.86)
- 3 month CG	134.53 (21.07)	133.95 (16.77)	134.24 (18.88)
- 6 month CG	134.66 (20.57)	131.78 (15.19)	133.26 (18.08)
-12 month CG	133.80 (19.97)	131.93 (15.79)	132.87 (17.87)
Brachial DBP, mmHg			
- Randomised	76.1 (7.81)	73.7 (8.64)	74.9 (8.27)
- 1 month CG	75.79 (7.86)	72.44 (8.16)	74.16 (8.13)
- 3 month CG	75.67 (8.00)	72.70 (8.41)	74.16 (8.29)
- 6 month CG	75.99 (7.89)	72.62 (8.21)	74.35 (8.16)
-12 month CG	74.95 (7.78)	71.89 (8.28)	73.42 (8.11)
PAT-AI, %			
- Randomised (32/30)	29.0 (21.19)	28.2 (16.30)	28.6 (18.83)
- 1 month CG (31/27)	27.91 (20.59)	29.46 (16.46)	28.64 (18.64)
- 6 month CG (28/25)	26.32 (17.71)	28.11 (15.12)	27.16 (16.41)
PAT-RHI			
- Randomised (32/30)	2.5 (0.91)	2.2 (0.61)	2.4 (0.79)
- 1 month CG (31/27)	2.50 (0.91)	2.19 (0.54)	2.36 (0.77)
- 6 month CG (28/24)	2.42 (0.78)	2.19 (0.54)	2.31 (0.69)
Data are expressed as mean (standard deviation). CIMT, carotid intima media thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; Tr, time to reflected wave; AI, augmentation index; AI@HR75, augmentation index corrected for heart rate of 75 bpm; PWV, pulse wave velocity; PAT-RHI, peripheral artery tonometry reactive hyperaemia index.			

Table C-2: Brachial artery SBP values at follow up visits according to treatment group

Time point (n, Allopurinol / Placebo)	Allopurinol	Placebo	P value
Baseline (40/40)	135.3 (20.3)	135.2 (18.4)	N/A
1 month (38/36)	128.7 (21.0)	128.4 (16.0)	0.54
3 months (35/36)	133.0 (19.8)	137.6 (15.6)	0.17
6 months (37/35)	130.2 (23.6)	133.3 (19.5)	0.16
12 months (30/30)	128.7 (22.4)	136.9 (21.0)	0.01
Data are mmHg, expressed as mean (standard deviation). P value refers to difference in change in brachial systolic BP from baseline (ANCOVA, adjusted for baseline brachial systolic BP)			

Table C-3: Brachial artery DBP values at follow up visits according to treatment group

Time point (n, Allopurinol / Placebo)	Allopurinol	Placebo	P value
Baseline (40/40)	76.1 (7.8)	73.7 (8.6)	N/A
1 month (38/36)	74.0 (7.5)	70.1 (9.7)	0.28
3 months (35/36)	79.1 (9.0)	78.1 (10.3)	0.85
6 months (37/35)	73.9 (10.0)	72.5 (9.4)	0.65
12 months (30/30)	73.0 (9.0)	72.4 (9.3)	0.47
Data are mmHg, expressed as mean (standard deviation). P value refers to difference in change in brachial diastolic BP from baseline (ANCOVA, adjusted for baseline brachial diastolic BP)			

Figure C-1: Dot plot illustrating mean maximum CIMT values at baseline (mm)

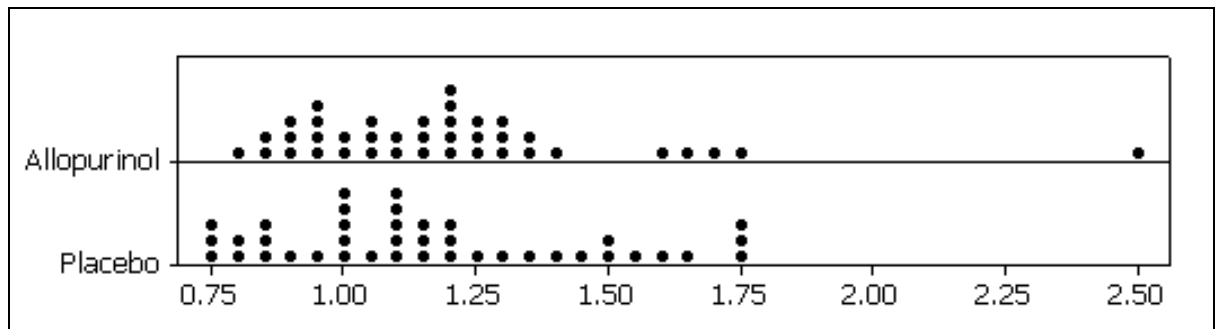


Figure C-2: Dot plot illustrating mean common (far wall) CIMT values at baseline (mm)

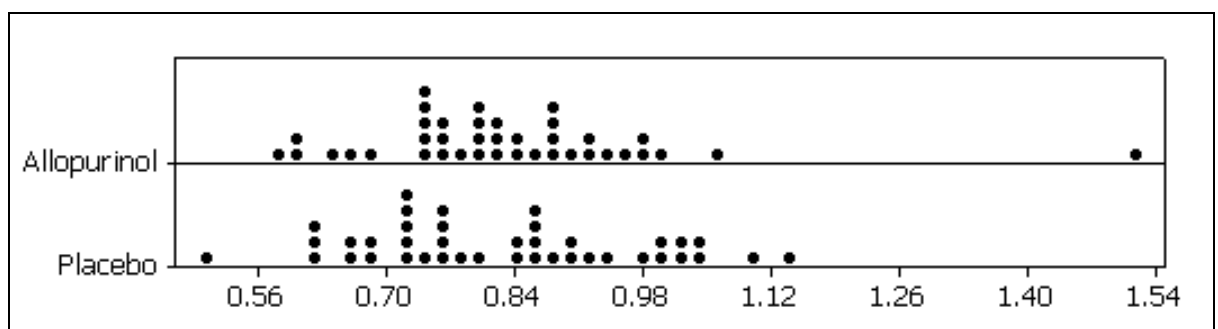
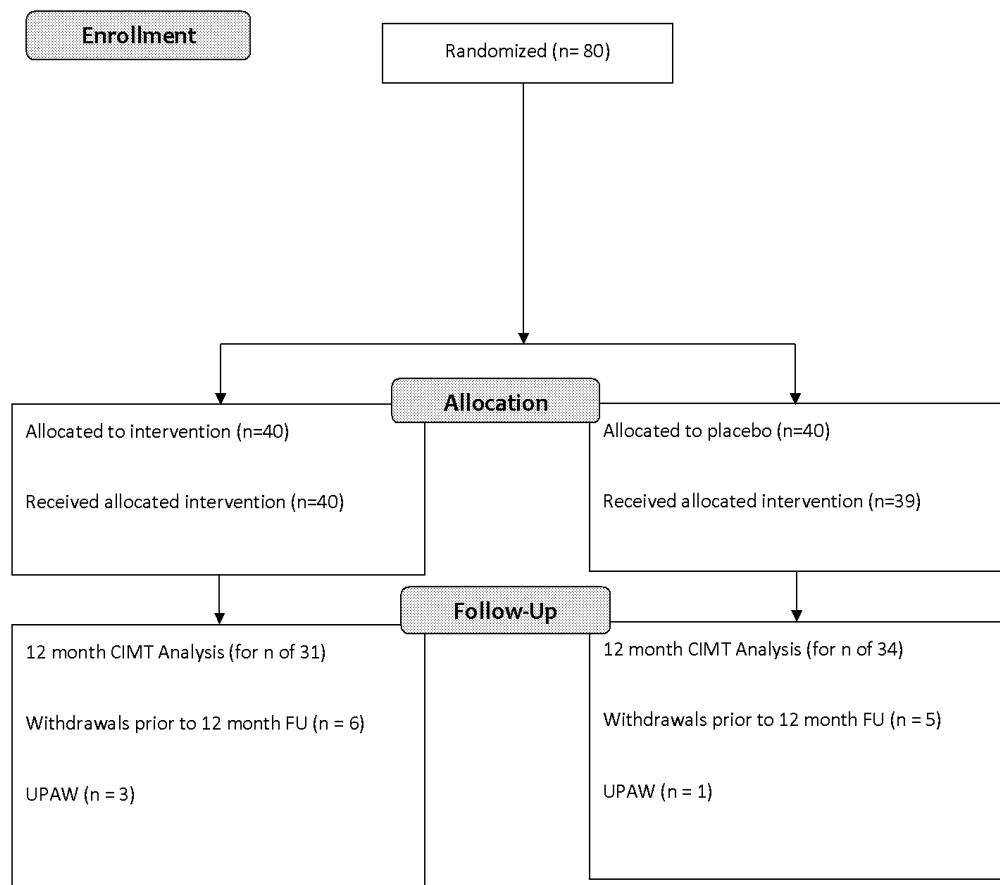
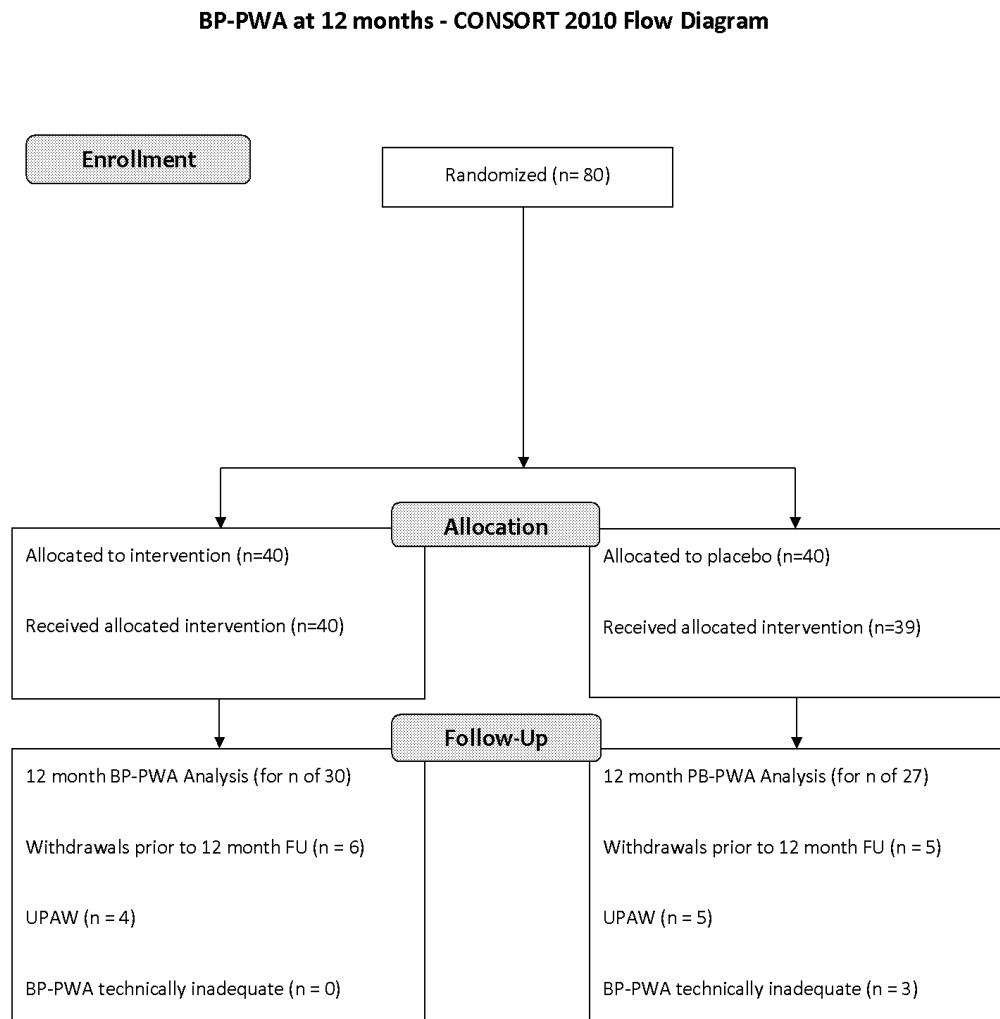


Figure C-3: CONSORT figure detailing unavailability of CIMT endpoint data at 12 months



CIMT, carotid intima media thickness; FU, follow up; UPAW, unavailability of patient to attend within protocol allocated window

Figure C-4: CONSORT figure detailing unavailability of BP-PWA endpoint data at 12 months



BP-PWA, Blood pressure pulse wave analysis; FU, follow up; UPAW, unavailability of patient to attend within protocol allocated window

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